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14. ABSTRACT
The Integrative Cardiac Health Project (ICHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. A translational research approach will provide the ability to find novel disease markers, optimal prevention and holistic treatment approaches, and a unique venue for future research as the "virtual laboratory" for optimal comprehensive health prevention in the military beneficiary population. This research method also allow us to further hypothesize and define relationships between CVD, other cardio metabolic disease states and maladaptive behavior patterns unique to service members such as pre-diabetes, stress, overweight and sleep disorders with the aim of targeting these disorders in a pre-clinical phase. Using an integrative, interdisciplinary preventive health approach, ICHP has shown that an individual's cluster of CV risk factors can be effectively targeted and improved.

15. SUBJECT TERMS- Lifestyle; Cardiovascular Disease (CVD); Prevention; Behavior; Coronary Heart Disease (CHD); Genomics; Metabolic Syndrome; PreDiabetes; Diabetes; Obesity; Stress

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Executive Summary - Integrative Cardiac Health Project
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The Integrative Cardiac Health Project (ICHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. In keeping with this aim, collaborative research efforts have continued between ICHP projects at Walter Reed National Military Medical Center, Windber Research Institute and Windber Medical Center. In the past year, the following key accomplishments are noted:

- Total yearly visits at ICHP WRNMMC/Windber Prevention Programs: 1811 (includes telephonic follow-up)
- Protocols completed -1; 5 protocols (including 1 sub study) in data analysis phase
- 4 active protocols in progress; 3 protocols in IRB Review
- Dissemination of scientific research findings continues:
  - 2 manuscript published; 1 in press; 1 submitted; 4 in preparation
  - 5 abstracts published; 4 abstracts presented as poster presentations
  - 2 accepted as podium presentations; 2 accepted as poster presentations
- Data analysis continues. Relevant findings in our population during the past year include:
  - BATTLE Study findings suggest knowledge of an abnormal CIMT and increased CV risk does not improve adherence to a lifestyle program
  - Analysis of AHA Ideal CV Health Risk Strategic Goals of CPP patients as an aggregate showed substantial improvement in all parameters.
  - There is evidence that important differences in levels of perceived stress, sleep quality and daytime sleepiness between white and black subjects in our program exist. These differences deserve explanation and may be valuable in designing interventions tailored for specific groups.
  - In a population with documented subclinical atherosclerosis and unremarkable conventional risk factor profiles, the ICHP CV Risk Score appeared to be more sensitive in identifying subjects at risk. The ICHP CV Risk Score may be a more discerning tool to guide risk reduction therapy in a prevention program.
  - A novel stress reduction technique, the ten-minute Tension Tamer, can reduce perceived stress levels in a majority of subjects resulting in improved sleep quality, decreased sleep latency and improved fatigue. The use of a portable stress reduction technique in short intervals may be a unique approach to improve cardiovascular risk through sleep improvement.
  - Intensive lifestyle modification can effectively alter CV risk factors, but successful weight loss may accentuate molecular change. Defining the role of weight loss in molecular response to lifestyle modification provides another dimension to understanding complex biological processes involved in CV health.
- Creation of new integrative, combination tracks with multiple aims to promote health and aggressively combat metabolic dysfunction
- Statistical support contract executed to enhance data analysis capabilities and dissemination of research findings
- Continue ICHP Database and Platform Creation: active building of infrastructure with robust plan for functionality and multiple protocol data capture capability

Introduction

The epidemics of cardiovascular disease (CVD), Type II diabetes, and obesity generate a major share of the preventable costs of American health care. Currently, the American health care market place does not support preventive care that would save lives and costs associated with these problems. Healthcare costs are predicted to rise from 16% of the US GDP in 2005 to 30% of the gross domestic produce by 2025 if we fail to invest in prevention. The primary vision of the ***Integrative Cardiac Health Project (ICHP)*** is to lead the way in CVD prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice in an effort to achieve the following research aims:

- Improve Force Health by better understanding the CVD risk susceptibility of military specific populations as well as to understand the individual service member through leading-edge research using novel tools and technologies.
- Investigate and create transformational models of healthcare delivery through personalized CVD prevention tracks as an adjunct to traditional care.
- Refine individualized prevention strategies through statistical data modeling to define the most cost-effective and sustainable approaches in promoting cardiovascular health throughout the military lifecycle.
- Simultaneously, improve understanding of the molecular, physiological, biochemical, immunological and environmental basis of cardiovascular (CV) health and disease and to use that understanding to develop improved approaches to disease diagnosis, treatment and prevention, in line with NHLBI Strategic Plan 2008.

Body

Overall Program Initiatives

On May 22, 2012, Dr. Vernalis briefed the Defense Center of Excellence (DCOE) Oversight Board chaired by Dr. Warren Lockette at the Military Health System (MHS). ICHP was supported by Dr. Lockette, but Dr. Vernalis was instructed to provide further support documentation from the Surgeon Generals, JTF CAPMED, and the Veterans Administration (VA). Since then, Dr. Vernalis has received approval from RADM Elizabeth Niemeyer, DCOE Representative for the Navy. In September, Dr. Vernalis participated in the Performance Triad Workshop, briefed LTG Patricia Horoho (Army Surgeon General) and received her full support. She presented to the Board of Directors at WRNNMC and was officially endorsed by RADM Alton Stocks. She has also briefed the Principle Deputy Undersecretary of VA Health, Dr. Robert Jesse who introduced her to Dr. O'Reilly, Director of Veterans Administration (VA) Research for potential synergies. Dr. Allison Haskell, Undersecretary of VA Benefits, and Dr. Patricia Hayes, VA Women's Health, are scheduled to visit ICHP in November and December. Dr. Vernalis is scheduled to brief her updates to the DCOE Oversight Board on Nov 20, 2012.

In addition, Meadville Medical Center in Meadville, Pennsylvania requested to use the ICHP Cardiovascular Disease Prevention Program (CPP) in a demonstration project to be conducted at Meadville and potentially Windber Medical Center. The possibility of this is being explored at HJF.

Core Award Tasks

Task #1: Completion of the "*B*etter Adherence *to T*herapeutic *L*ifestyle Change *E*fforts (BATTLE) Trial".

Study Methodology

The purpose of this study is to determine whether knowledge of abnormal results from a noninvasive test for detection of subclinical atherosclerosis (CIMT), in addition to knowledge of CVD risk factors, enhances adherence to healthy lifestyle behaviors in comparison to only CVD risk factor knowledge. The study will be conducted with individuals at moderate to high risk for cardiovascular events based on CVD risk factor profile and evidence of significant subclinical atherosclerosis.

This two-arm, double-blinded study will randomize subjects to either receive CIMT results (R-CIMT Group) or have CIMT results withheld (W-CIMT Group) in the setting of a 3-month TLC intervention. After the 3-month TLC intervention period is completed, subjects who had CIMT results withheld will receive this information. Because knowledge of the study hypothesis could impact their behavior during the lifestyle intervention, subjects will be blinded to the study hypothesis. Similarly, research staff implementing the TLC intervention will be blinded to subjects' randomization assignment.

It is hypothesized that participants with CVD risk factors who have knowledge of their own CIMT test results showing significant subclinical atherosclerosis will demonstrate better adherence to TLC than those subjects from whom the CIMT test information is withheld. A composite index of adherence to the TLC intervention was selected as the primary outcome measure since the main goal of this study is to assess the impact of CIMT imaging knowledge on change in lifestyle behaviors.

A combined measure of adherence, reflecting both aspects of the lifestyle intervention (Mediterranean-type diet, moderate aerobic exercise), was chosen that uses accepted measures of diet and exercise adherence reported in the literature. Secondary outcomes include: 1) Adherence to each program components; 2) Changes in modifiable CVD risk factors and other biochemical markers; 3) Emotional factors such as anxiety, self-efficacy, motivation, and 4) Atherosclerosis and CIMT Knowledge Assessment Score (only in CIMT-R subjects).

Results:

Of the 1068 interested participants contacted about this study, 441 (41%) were consented and screened for participation in this study, 11% were ineligible due to low cardiovascular disease (CVD) risk profile and 47% opted out primarily for study time commitment and travel/distance reasons. Of those consenting participants, 275 (62%) screened out for the following reasons: 60% had CIMT <75 percentile for gender/age, 14% had an unacceptable past medical history, 11% withdrew consent, 6% did not meet other diagnostic or severity criteria, 1% had an intercurrent medical event during screening, and 8% were categorized as other (deployment, relocation, job conflicts). In summary, approximately only 18% of those patients who met initial screening criteria after the telephone screen (n=948) randomized into the main study.

The remaining 166 were randomized to treatment group (83 per group); receive CIMT (R-CIMT) vs. control did not receive CIMT (W-CIMT). Thirty study cohorts yielded 142 completers vs. 24 non-completers (14.5% dropout rate); however, 161 participants had at least one clinical observation after study randomization and were included in an intent-to-treat analysis. Reasons

for non-completion were: withdrew consent (4.2%), protocol non-compliance after randomization (3%), adverse events (2.4%), lost to follow-up (1.8%), and other (3%).

Study completers were predominately middle aged (mean=54.7 yrs; range 26-78), overweight (mean BMI=31.5 ± 5.6), Caucasian (48%), females (64%); however, statistical significant differences in the study groups were detected in mean age and gender. The treatment group was older (56.8 ± 9.4; p=0.015) and comprised of more women (74%; p=0.018). No differences between groups was detected in overall reported co-morbid conditions, however, over 50% of the women in the treatment group were postmenopausal. Completers were 53% hypertensive, 82% dyslipidemic, 12% Type 2 diabetes, 4% current smokers, and 56% with family history of CVD. No differences were detected between the treatment groups in their CVD risk profile.

Since assessing the impact of CIMT imaging knowledge on change in lifestyle behaviors was the primary study goal, a composite index of adherence to the lifestyle program intervention was selected as the primary outcome measure. A combined measure of adherence, reflecting both aspects of the lifestyle intervention and that uses accepted measures of diet and exercise adherence reported in the literature, was chosen. At study closeout, both groups showed marked improvement in both % of diet and exercise adherence change as compared to baseline, however, no difference was detected between the study groups ([R-CIMT] =19.6 ± 24.3 vs. [W-CIMT] = 22.6 ± 24.2); p=0.519), thereby, confirming the null hypothesis that knowledge of an abnormal CIMT scan did not have a motivational impact on overall adherence to the TLC intervention in this study.

Although the hypothesis was not supported, study completers did make significant improvements in most of their modifiable risk factors (anthropometrics; total and LDL cholesterol; triglycerides. Slight increases were seen in systolic and diastolic blood pressure. Measures of obesity including weight, BMI and % body fat were reduced by 5%. Additionally, a 5% reduction in waist circumference and a 7% reduction in abdominal sagittal diameter were seen. Both systolic and diastolic blood pressure increased by 2%. Levels of total cholesterol were reduced by 6%, LDL-cholesterol decreased by 9% and triglycerides were lowered by 14%. C-reactive protein (CRP) was decreased by 17%. Despite these positive changes, a 1% reduction in HDL-cholesterol was seen. Serum fasting glucose and insulin were collected and HOMA scores calculated as a measure of insulin resistance (IR). At baseline, 48% of the study completers had HOMA score > 2.8, indicative of IR. At study completion, 19 subjects were able to lower their HOMA scores < 2.8 and reduce their risk of pre-diabetes. Overall, serum glucose was reduced by 4% and fasting insulin was reduced by 23.3%.

Although these data do not support the motivational impact of CIMT imaging on program adherence, it is clear that this data supports participation in a multi-faceted lifestyle change which includes intensive education, frequent monitoring and group support. Participation resulted in substantial CVD risk factor improvements. Some of these changes rival what has been observed with pharmacological treatment.

In addition to the main study, a formative evaluation of the lifestyle program intervention took place almost 2 years after study completion for many study participants. Of the 140 surveys mailed to consenting BATTLE Study participants, 49% (n=68) were returned. Additionally, 35 telephonic interviews (31 year 2 completers; 4 non-completers) were conducted on consenting participants over a 2 month period. Survey item responses have been collated and all telephone interviews have been transcribed. Identification of common theme from telephone interviews and open-ended survey response continues.

Protocol Deviation: One protocol deviation was reported to the WRAMC DCI Human Use Committee during this study and previously reported.

Adverse Events: During the course of this study, 10 serious (SAEs) and 25 non-serious AEs have been reported to WRAMC DCI Human Use Committee. A summary of AEs has been previously reported.

Status: The annual Continuing Review (CR) was approved by WRNMMC Department of Research Programs (DRP) on 28 Nov 11 and forwarded to MRMC. This study is closed to accrual and data collection/analysis is complete on main study. Qualitative analysis for formative review continues. All final study documentation was received by PREMIER CRO in Dec 11. Study will be closed on next CR due to DRP by 28 Oct 12. Manuscript preparation and submissions to continue.

The following abstract was *accepted* as a podium presentation poster at a national meeting (See Appendix A):

- Modlin RE, Walizer EM, Vernalis MN. CIMT imaging knowledge effect on lifestyle program adherence. TriService ACP, Bethesda, MD, 1-3 Nov 12.

Manuscript *submitted* (See Appendix A):

- Saum NS, Halsey JF, Walizer EM, Vernalis MN. Exploring the role and impact of limited mindfulness training in changing diet and exercise behaviors. (Resubmit in preparation).

Manuscripts *in preparation*. Planned submission - next quarter:

- Walizer, EM, Vernalis, MN. Methodology and demographics of the BATTLE Trial (Better Adherence to Therapeutic Lifestyle Change Efforts). (In preparation)

- Walizer EM, Vernalis MN. Does visual knowledge of increased risk for cardiovascular disease affect lifestyle change program adherence? (In preparation)

Task #2: Completion of the CADRe Five-Year Follow-up Protocol.

Methodology

This follow-up study will determine the persistence of healthy lifestyle behavioral changes and CVD risk factor control results after their original CADRe study participation. This study will continue as a longitudinal observational study where patients will have yearly follow-up visits at 1, 2, 3, 4, and 5 years after completion or expected completion of the CADRe Study. This study will involve prospective collection of data. All collected data is considered WRNMMC Cardiology standard of care for the study population identified.

It is hypothesized that participants who have been exposed to an intensive lifestyle change program will demonstrate long-term carryover of heart healthy characteristics including persistence of favorable lifestyle change behaviors and risk factor control. Up to 163 male and female CADRe study participants, age 18 years or older, with subsequent completion of Phase 1 of the CADRe Study (3-month data collection) were recontacted and invited to participate in this 5-year follow-up study (post-study completion or expected completion).

A composite index of 7 heart healthy characteristics (BMI 18.5 – 25; LDL-cholesterol < 100 mg/dL; dietary fiber intake ≥ 25 gms/day; consumption of 5 or more fruits and vegetables per day; BP < 140/90 mmHg; regular exercise ≥ 150 min/week, and daily practice of CADRe

program stress management techniques) was selected as the primary outcome measure since the main goal of this study is to assess the persistence of lifestyle change behaviors and risk factor control. The Heart Health Index (HHI), presented as a single score (range 0-7), will be assigned to each subject yearly. Additionally, each of the 7 heart healthy characteristics will be assessed independently as a continuous variable. Secondary outcome measures include: Changes in modifiable CVD risk factors (blood pressure, body composition and fitness, lipid levels and glucose); C-reactive protein and, Quality of Life.

Preliminary Results:
Of the 163 eligible CADRe study patients, 76 provided informed consent and made at least 1 follow-up visit. Preliminary analysis of changes in modifiable CVD risk factors (BP, body composition and fitness, lipid levels and glucose) has already been reported in previous reports. In 62 participants with Year 5 data, there were significant increases in both body composition and systolic BP when compared to final CADRe study visit data. Body anthropometrics show an 8% mean weight gain and a 22% increase in body fat despite reporting a mean of 175 minutes per week of moderate physical activity. Systolic BP increased by 4%. No significant change was seen in diastolic BP, glucose, HDL or CRP. However, significant reductions in TC, LDL and TG at Year 5 were 4%, 4% and 10%, respectively. Of the 56 participants on lipid-lowering medications, 93% reported either no change (n=29) or an increase (n=23) in these medications which may account for the lipid profile changes.

Table 1. Select Outcome Variables at 5-Year vs. Final CADRe Study Visit (n=62)

	Final CADRe Study Visit	5-Yr Follow-up Visit	Change	P
Body Composition/Blood Pressure (BP)				
Weight (kg)	82.5 ± 22.6	89.2 ± 25.3	6.7 ± 9.9	<0.001
BMI (kg/m^2)	27.6 ± 6.1	30.2 ± 6.7	2.6 ± 3.6	<0.001
% Body Fat*	26.4 ± 9.1	31.2 ± 9.2	4.8 ± 4.3	<0.001
Systolic BP (mmHg)	120.8 ± 12.4	125.4 ± 14.7	4.6 ± 14.2	0.014
Diastolic BP (mmHg)	69.1 ± 7.3	70.9 ± 7.7	1.8 ± 8.9	0.121
Laboratory (mg/dL)				
Glucose (mg/dL)**	96.8 ± 15.7	96.2 ± 17.9	-0.7 ± 16.5	0.746
Total Cholesterol (mg/dL)	158.9 ± 31.3	150.3 ± 30.1	-8.6 ± 29.9	0.027
LDL-Cholesterol(mg/dL)	87.1 ± 23.2	81.5 ± 24.5	-5.6 ± 21.6	0.046
HDL-Cholesterol(mg/dL)	46.1 ± 10.2	48.7 ± 13.1	2.6 ± 11.2	0.076
Triglycerides (mg/dL)	157.7 ± 88.8	130.0 ± 84.5	-27.8 ± 75.5	<0.001
C-reactive protein (mg/dL)#	0.226 ± 0.275	0.242 ± 0.412	0.016 ± 0.379	0.479

Values are mean ± SD; *n=57; **n=61; #n=60.

Final analysis in CADRe study completers comparing follow-up data from Year 5 compared to 2 baseline time points (CADRe Baseline and study completion) is pending.

Adverse Events: There have been 2 adverse events (AEs) reported to the WRAMC HUC during the course of this study and previously reported.

Status: The CR was approved by WRNMMC DRP on 19 April 2011 and forwarded to MRMC for review; approval letter still pending. This study is closed to accrual and data collection is complete. Data reconciliation and final codebook were completed. Statistical support contract with CLINIRX was executed and data then forwarded to CLINIRX for analysis. Final data tables

have been received and are under review. Plan study closure once final data analysis is received. Manuscript submissions to begin in the next quarter.

Task #3: Continuation of the "Comprehensive Cardiovascular Health Risk Assessment and Prevention Center (CPC)".

Methodology

This program serves as a platform for ongoing translational research activities, a "virtual laboratory" based on scientific findings for the development of best personalized preventive practices. In other words, the platform allows ICHP to gather an expansive number of data points for each patient or subgroup of patients (eventually combined with data at a molecular level) that when leveraged will result in the creation of new tools in technology to define wellness, predict and prevent disease, and empower patients and providers to transform their healthcare.

The CPP platform has a dual purpose and is multifunctional. This platform 1) allows for multiple research protocols to be conducted as it sets the stage for recruitment, enrollment and hypothesis generation, advanced data modeling and simultaneously 2) provides a venue where research findings from these protocols can then be tested, validated and translated into application for clinical practice. Our protocols within the CPP are specifically designed to examine the effects of our military's high op tempo which predisposes our service members to accelerated atherosclerotic risk resulting from high stress, PTSD, depression, sleep insufficiency, overweight, prediabetes and prehypertension among other traditional disease risk factors.

This program was established to address the unique needs of military beneficiaries at risk for CV disease. It includes conventional and novel CV risk profiling (health assessments, labs, markers, wearable monitors) and tailored, personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a cardiologist, sleep specialist, nurse practitioners, nutritionists, stress management instructors and exercise physiologists. Validated tools to screen for and measure CV risk are part of this inclusive package. Report cards for the patient and provider as well as email notifications are utilized. The program is an adjunct to the best medical practices provided by their primary care provider. Up to 1000 patients may be enrolled each year. Some of the patients (such as nurses or traumatic injury patients, etc) may be in subgroup programs because of unique needs. The CPP serves as a platform for ongoing translational research activities, a "virtual laboratory" for the development of best preventive practices and for CV educational and marketing materials.

The "Outcomes of the CPP Program" protocol provides for retrospective examination of existing data for the purpose of examination and reporting of the results of the evaluations and interventions of the CPP. The CR was approved 27 Mar 12.

Status:

-Total patient visits: 885 + 470 telephonic visits = 1,355 in past year
-Customer satisfaction surveys continued to average a score of 3.97 out of 4.0, demonstrating high patient satisfaction despite our relocation to the WRNMMC Bethesda campus.

Manuscript *published* (See Appendix A):

- Kashani M, Eliasson A, Vernalis M. Perceived stress correlates with disturbed sleep—a link connecting stress and cardiovascular disease. *Stress:* the International Journal on the Biology of Stress. 2011;19 June [epub ahead of print]. (In print - 2012;15(1):45-51.)

Abstracts published (See Appendix A):

- Eliasson A, Kashani M, Vernalis M. Fatigued on Venus, sleepy on Mars? Am J Respir Crit Care Med 2012;185:A5033.

- Kashani M, Eliasson A, Bailey K, Vernalis M. Novel tool improves CV risk stratification and guides therapy. Circ Cardiovasc Qual Outcomes. 2011;4:AP88.

- Eliasson A, Kashani M, Hoffman J, Vernalis M. Racial differences in perceived stress, sleep habits, and daytime symptoms. Sleep 2011;34:A262.

The following abstracts were ***presented* as posters at national meetings in the past year** (See published abstract above):

- Eliasson A, Kashani M, Vernalis M. Sleepy on Venus, Fatigued on Mars? American Thoracic Society, 22 May 2012, San Francisco CA.

The following abstracts were ***accepted* as podium presentations at national meetings in the past year (See Appendix A):**

- Kashani M, Eliasson A, Bailey K, Vernalis M. Novel stress reduction technique improves sleep and fatigue. American College of Chest Physicians, 22 Oct 12, Atlanta, GA.

1st quarter (29 Sep 11 – 29 Dec 11):

- Total patient visits 230 = Sep 11-Dec 11.
- Revised all data collection forms for providers and patients: toolkit, referral forms, and educational sheets.
- Implemented "integrative synthesis system" for multidisciplinary clinical review team for objective and subjective biometric data review.
- Created of new multidisciplinary initiative to translate research into practice; Setting the Stage for Success for all domains of the program-anticipating patient needs (pre-emptive and proactive)
- Continued ICHP Database and Platform Creation: Patient and provider modules designed; workflow and process of clinical/research milestones outlined for intuitive navigation of application.
- Traumatic Amputee Soldier Protocol received final approval with ICHP involvement in wounded warrior evaluation. Ready to assess soldiers and assess health status.
- Proceeding successfully with Statistical Analysis contract finalization for ICHP's research initiatives.
- Workshops at WRNMMC site continue to be well attended. Appointments being made 2 months in advance. Working on new marketing initiatives as visibility on base is key to patient enrollment.
- **Overcoming significant barriers encountered upon move to new location in August 2011. Still experiencing issues with lack of parking for patients and staff--leading to loss of patients and personnel. ICHP is now functional with a full appointment schedule and doors open for participants.

2nd quarter (30 Dec 11-29 Mar 12):

- Total patient visits 260 = during Dec 11- March 12.
- Updating marketing materials for future recruitment initiatives; to include hospital banners, signs, patient brochures, provider referral forms

- Multidisciplinary initiative to use patient's motivational status in an algorithm as a way to prescribe lifestyle practice; allowing for a spectrum of care for patients at different stages of change
- Continued ICHP Database and Platform Creation: Patient and provider modules designed; workflow and process of clinical/research milestones outlined for intuitive navigation of application. Expansion of patient modules to include the possibility to receive new alerts from the clinical team.
- Proceeding successfully with Statistical Analysis contract finalization for ICHP's research initiatives. Including 5 customized queries each quarter to be reported to ICHP leadership focused on productivity and clinical outcomes.
- 3 Empowerment Workshops conducted and well attended. Appointments being made 2 months in advance. Expansion of food demonstrations to provide patients with practical solutions and ideas to improve the quality and quantity of food in spite of stress eating and diabetes.
- ** Still experiencing issues with lack of parking for patients and staff--leading to loss of patients and personnel. ICHP is now functional with a full appointment schedule and doors open for participants.
- Hiring /orientation of new personnel: Nurse Executive, Nurse Practitioner, Sonographer. Recruitment in process for Stress Management Instructor.

3rd quarter (30 Mar - 29 Jun 12):

- Total patient visits 395 = during Apr 12 – Jun 12.
- Continuation of creating new marketing strategies for future recruitment initiatives including hosting several WRNMMC departments (Endocrine and Cardiology Services) for information workshops
- ICHP featured in WRNMMC's *Journal* with "Heart Health Focus of Program of Excellence at Walter Reed Bethesda" article and Fort Gordon's *The Signal* as a program that helps to improve the lives of military beneficiaries.
- Creating new integrative, combination tracks with multiple aims, i.e. Stress Eating, using stress reduction, sleep expansion and nutrition
- Continued ICHP Database and Platform Creation: Patient and provider modules designed; workflow and process of clinical/research milestones outlined for intuitive navigation of application. Laying the foundation for the sustainment portion of the program- focus on outreach
- Statistical Analysis contract finalized. Data collection and communication plans agreed upon.
- Patient enrollment showing gradual increases as ICHP offers patients a flexible appointment scheduling option given the patient parking obstacles at new location

4th quarter (29 Jun- 29 Sep 12):

- Total patient visits 460 = during 29 Jun- 29 Sept.
- Continuation of new marketing strategies for future recruitment initiatives including collaboration with Executive Medicine and Medical Home.
- Highlighting high risk factors not commonly used risk scores; Family history of premature CVD (high risk factor) to patients: 1. Specific assessment and tracking 2. Personalized patient handout informing of risk and 3. initiation of Self Efficacy Survey to measure patient's ability to make healthy choices when at high risk for CVD
- Creating new integrative, combination tracks with multiple aims, i.e. Stress Eating, using stress reduction, sleep expansion and nutrition clinical pathways

- Continued ICHP Database and Platform Creation: Patient and provider modules designed; workflow and process of clinical/research milestones outlined for intuitive navigation of application. Establishing a mechanism to generate lifestyle prescriptions by compiling ICHP data assessment and expertise that will serve as potent motivators to keep patients on track and adhere to healthy living regimen
- Statistical Analysis: Data files de-identified and outcome variables identified for data dictionary
- Responding to new interest from Veterans Administration in program model and success
- Analysis of AHA Ideal CV Health Risk Strategic Goals of CPP patients as an aggregate showed substantial improvement in all parameters

Analysis of AHA Ideal CV Health Risk Strategic Goals of CPP patients as an aggregate showed substantial improvement in all parameters from baseline to program completion

Smoking (quit > 12 mo)		Total Cholesterol (< 200)		Blood Pressure (< 120/80)	
17	7	253	279	43	79
394	394	394	394	394	394
0.04	0.02	0.64	0.71	0.11	0.20
Improved 53%		**Improved 11%**		**Improved 82%**	

BMI (< 25)		Sleep (> 7 hrs)		Exercise (IPAQ ≥ 500)	
67	85	156	171	206	277
393	392	391	372	393	393
0.17	0.22	0.40	0.45	0.52	0.71
Improved 29%		**Improved 15%**		**Improved 37%**	

Stress (PSS < 23/56)	
196	251
341	334
0.57	0.75
Improved 32%	

Sub Task #3.1 Completion of the "Validation of the ICHP Cardiovascular Risk Score" protocol.

Methodology

Data previously collected on patients enrolled in the Prospective Army Coronary Calcium (PACC) and PACC Rescan projects were reviewed. Specific information was gathered and analyzed to give each patient a CV disease risk score according to a formula developed by the ICHP. This ICHP formula uses the Framingham model of risk prediction and adds historical factors and biochemical markers to produce a novel score predictive of CV disease risk in military beneficiaries. The goal of the study was to validate the utility of this novel ICHP scoring system by comparing the predicted risk with outcomes in this well characterized population. The primary objective of the project was to validate the predictive utility and accuracy of the ICHP CV risk score (or ICHP score). Specifically, the goals: a) to determine if the ICHP score correlates with cross-sectional prevalence of coronary calcium as measured in the PACC project and b) with the development of CHD events such as angina, myocardial infarction, or need for CV intervention such as coronary stenting, angioplasty, or bypass surgery. A third goal:

c) to determine the correlation of the ICHP score with coronary calcium progression as measured in the PACC rescan project.

Status: After statistical analysis of data from the PACC project, ICHP score performed successfully in the linear model with a coefficient of 0.003 (p=0.004), indicating that an increase of one point in ICHP score was associated with increasing CIMT 0.3%. In the logistic model, the odds ratio for the ICHP score was 1.04 (p=0.01), signifying that a one point increase in ICHP score increases odds by 4% of having a top quartile "atherosclerosis score". In conclusion, incorporating novel risk factors such as those proposed in the ICHP score and considering the value of family history may significantly improve the predictive accuracy of CVD risk assessment and may reveal appropriate targets for therapeutic intervention.

These findings emphasize the need for improved CV disease risk identification in women. Family history and other novel risk factors add predictive value to current risk models and identify potential therapeutic targets. Analysis to continue with data set from ZENITH study.

Manuscript (in final submission):
- Systematic inquiry of family history improves cardiovascular disease risk estimation.

Sub Task #3.2: Initiate the "ZENITH (randomiZed Evaluation of a Novel comprehenslve prevention program on aTHerosclerosis progression) Trial".

Methodology
The purpose of this one-year, prospective, randomized, controlled, interventional trial is to investigate the impact of ICHP-CPP on vascular health, atherosclerosis progression and left-ventricular relaxation (diastolic function) among patients with increased lifetime CVD risk, but low short term coronary heart disease (CHD) risk (according to the Framingham Risk Score, FRS) as compared to receiving usual care (UC). Up to 170 male and female patients between 18-50 years of age with low (<10%) 10-year FRS for CHD but estimated lifetime risk (to age 95 years) of coronary death or myocardial infarction (MI) of ≥ 39% without clinically manifest CVD [MI, coronary or peripheral arterial revascularization, obstructive coronary artery disease (CAD), heart failure or cerebrovascular event] will be randomized to participation in the currently ongoing ICHP-CPP or to UC. The primary endpoint is between-group differences in the change in vascular endothelial function as measured using DTM, as reported as adjusted. Secondary endpoints are changes in measures for CIMT, cardiac diastolic function, lifetime CHD risk scores, and the ICHP CV Risk Score. It is hypothesized that patients with low-short term (Framingham 10-year CHD risk score) but high lifetime estimated risk for coronary death or MI who participate in the ICHP-CPP will improve vascular health and reduce atherosclerosis progression when compared to those receiving usual care.

Status: Protocol submitted to WRNMMC DRP 10 May 12. Administrative revisions completed on 25 Jun 12. Received WRNMMC Scientific approval on Aug 12. Planned IRB review on 8 Nov 12. Statistical support contract executed for this protocol – reviewed methods and case report forms.

Sub Task #3.3: Development of ICHP CPC Prospective Registry.

Methodology:
The purpose of this study is to establish a registry to enable research on patients at risk for cardiovascular disease (CVD). All clinically derived patient-related data for subjects participating in the WRNMMC CPP will be entered into a single, secure database. At periodical

intervals, assessment of the registry database will allow queries to define the impact of an integrative lifestyle change program on CVD risk over time. The ICHP Registry will utilize the ICHP database which documents demographics, responses to validated lifestyle habits questionnaires regarding exercise, diet, stress and sleep, physical examination and anthropometrics, laboratory test results, imaging, actigraphic data, clinical recommendations and consultations, participant management, and participant visits.

Patients will be offered enrollment into this study at the time of presentation if they are military health care beneficiaries and are at least 18 years of age. All participants, regardless of enrollment in the study, will receive the usual standard of care by their health care providers. Collection of medical information on ICHP subjects is accomplished through interview of patients as well as through review of medical information from other facilities providing care. Clinical data collection occurs at baseline and at the conclusion of the intervention, typically at 6 months. Additional follow up for support of the patient's gains and additional data collection occur at 12 months and annually for up to 5 years. The research component of this study will involve the analysis of clinical data collected at these intervals.

The ICHP clinical database can be queried at a single sitting with removal of all personally identifying information to perform assessments of prevalence of risks, associations of behaviors and risks, and the success of various interventions over time. Such queries take minutes to perform and can be accomplished with minimal risk to individual privacy. There is no need to maintain any linkage data as the information is harvested at a single sitting from one database requiring no marriage with external data sets.

Status: Protocol submitted to WRNMMC DRP on 13 Dec 11. Protocol received WRNMMC Scientific Approval on 28 Sep 12. Planned WRNMMC IRB review on 8 Nov 12.

Sub Task #3.4 Collaboration on "Assessing Risk Factors for Cardiovascular Disease in Individuals with Traumatic Amputations" protocol (PI: LTC Anne Andrews, SP, PhD, WRNMMC Dept of Ortho & Rehab).

Methodology:
The objective of this comparative cohort study is to assess presence of known risk factors for CVD in individuals with traumatic amputations. Up to 405 participants will be enrolled and divided into three groups: no injury, traumatic orthopedic injury with amputation, traumatic orthopedic without amputation. Data will be collected at two time points, at time of consent and at a 5-year follow-up visit, and will include demographic (including diagnosis of hypertension, hyperlipidemia or diabetes mellitus) and family history, anthropometric (height, weight, waist circumference, hip circumference and body composition), biochemical (lipids, fasting blood sugar, hemoglobin A_{1c}, fasting insulin, ultra sensitive C - reactive protein, lipoprotein (a), thyroid stimulating hormone, vitamin D, and fibrin D-dimer), blood pressure, heart rate, pulse pressure, EKG, carotid intima-medial thickness (CIMT) study, stress and sleep surveys, diet (fruit and vegetable intake, total fat and saturated fat intake), smoking history and activity measures. CVD risk will be estimated using the Integrated Cardiac Health Project (ICHP) risk assessment and the National Heart Lung and Blood Institute (NHLBI) 10-year risk estimate. It is hypothesize that: 1) Individuals with traumatic amputations (A) will have higher levels of factors that increase risk (anthropometry, biochemical markers, blood pressure, pulse pressure, CIMT, stress, poor sleep habits, saturated fat intake, smoking) and lower levels of factors that decrease risk (fruit and vegetable intake and activity) for CVD when compared to individuals without orthopedic injuries (N), and that this risk will continue to increase over the 5-year follow-up; 2) Individuals with traumatic amputations (A) will also have the same increased risk factors, as stated above, when compared to individuals with traumatic orthopedic injuries that did

not result in amputation (O), and again this risk will continue to increase over the 5-year follow-up, and; 3) There will be no difference in presence of risk factors between individuals with (O) and without orthopedic injuries (N), that did not result in amputation.

Status: LTC Andrews received WRNMMC IRB approval on 23 Feb 12 and begun study enrollment/data collection in March. ICHP personnel involvement (collection of EKG, CIMT, training on questionnaires scoring guidelines) began upon MRMC HRPO approval on 10 Aug 12. An amendment has been submitted to WRNMMC DRP for Inclusion of extramural funding source and submission of MRMC HRPO Approval Letter. Current enrollment: 25.

Task #4: Initiate "Lifestyle Education and Support Empowering prevention in breast disease patients (LEASE) Trial.

Status: Protocol in development

CSI Award Tasks

Task #1: Follow-up data analysis and publication for Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal and the Sub-Study for Subjects in the Dr. Dean Ornish Program protocols (ICHP Windber Research Institute (ICHP-WRI).

Methodology

This study will examine DNA polymorphisms and genes/proteins with altered expression during a lifestyle change program designed to stabilize or reverse CHD. DNA, RNA, and protein from individuals participating in the lifestyle change program (as well as matched controls) will be analyzed for qualitative and quantitative differences using high-throughput technologies that permit genome-wide profiling. Polymorphisms (SNPs) will be determined from DNA in peripheral blood and associations of these polymorphisms with response will be investigated. Gene and protein expression will be determined at the beginning of the program (baseline), after 12 weeks, and after one year in each individual. Differential expression will be defined as expression levels (RNA or protein) that change 2+ fold from baseline to 12 weeks, from baseline to one year, or from 12 weeks to one year. Differentially expressed genes/proteins in Ornish program participants will be compared with those in the control individuals who do not participate in the Ornish program to identify genes that may be important in CHD reversal and/or progression.

We hypothesize that intensive lifestyle changes involving diet, exercise, meditation, yoga and group support, which lead to improved CHD risk factor profiles and arteriographically-documented coronary artery disease reversal or stabilization, will positively correlate with changes in expression for genes associated with lipid metabolism, protein biosynthesis, protein modification, transcription regulation and/or cell surface receptors.

Status:

Enrollment to the global profiling study is closed and all active participants have completed their participation in the study. Data analysis is ongoing.

Subject Enrollment and Demographics

Subject enrollment was 374. There were 166 participants taking part in the lifestyle change program, 140 subjects serving as the control group, and 68 participants enrolled in the Sub-study. Demographic characteristics of the control group were: average age of 63.7 years, 51%

were female, 29% were veterans or the spouse of a veteran, and 34% had diagnosed coronary heart disease.

Data:

Inflammation biomarker panel – Findings reported previously.

Manuscript *accepted* (See Appendix A):

- Voeghtly LM, Neatrour DM, Decewicz DJ, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. Improvement in cardiometabolic risk factors during an intensive cardiovascular lifestyle intervention. Nutr Metab Cardiovasc Dis 2012 (*in press*).

Macrophage migration inhibitory factor (MIF) – MIF is an inflammatory cytokine that regulates smooth muscle cell migration and proliferation, and thus plays an important role in promoting development of atherosclerotic lesions. MIF has been shown to be an important biomarker for diseases with inflammation, such as CVD, diabetes, obesity, and cancer.

Genotyping of genetic variants in MIF gene that influence circulating levels completed; data analysis complete. MIF levels decreased significantly ($p<0.05$) in Ornish participants compared to controls at 12 weeks, but no difference in MIF levels between cases and controls at one year. Only women participants showed significant ($p<0.05$) reductions in MIF levels at 12 weeks (-23%). No change in men. Transcription of the human MIF gene is regulated by genetic polymorphisms in the MIF promoter, including the –173G/C single-nucleotide polymorphism and a sequence of tetra-nucleotide repeats at –794 (-794CATT$_{5-8}$). These polymorphisms may have relevance to cardiovascular disease, and this area has become a growing area of investigation; however, the tetranucleotide polymorphism and SNP variants were too infrequent for meaningful analysis.

Abstract *accepted* for poster presentation (See Appendix A):

- Miller EJ, Mamula KA, Leng L, Piecychna M, Vernalis MN, Bucala R, Ellsworth DL. Cardiovascular disease risk factor modification decreases HS-CRP and Macrophage Migration Inhibitory Factor (MIF): Influence of gender. American Heart Association Scientific Sessions 2012, 3-7 Nov 12, Los Angeles, CA.

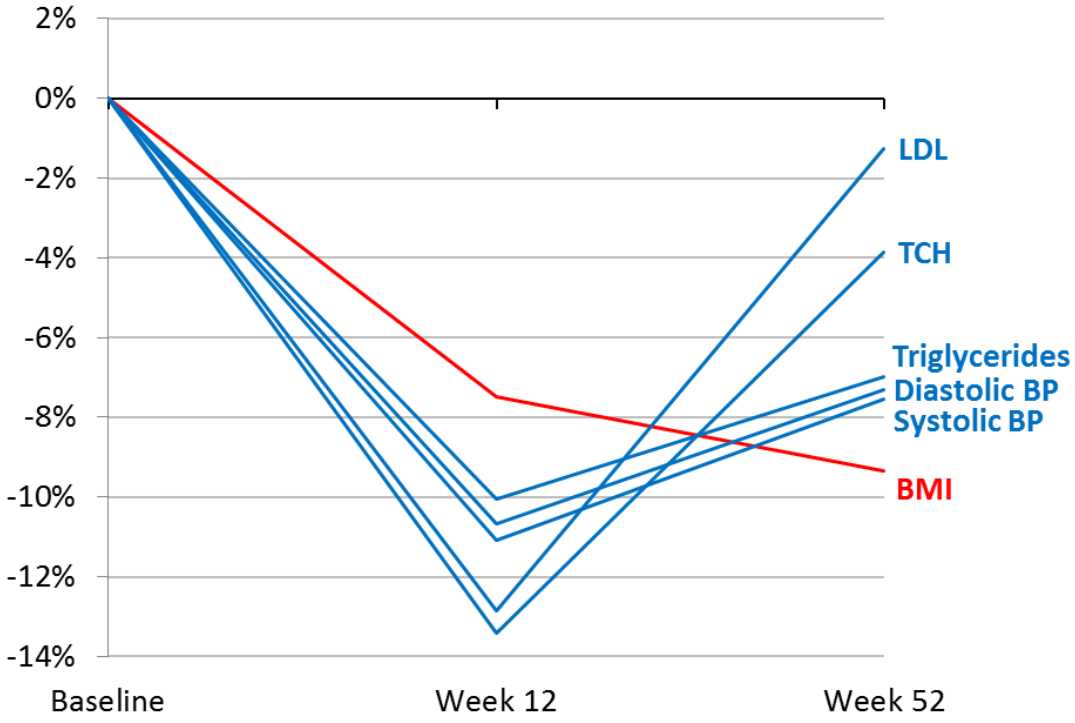
Gene Expression – Pathway analysis was performed on Ornish vs Control datasets, comparing each time point vs control, using three different pathway databases, KEGG, BioCarta, and Broad Molecular Signature Data Base. For the BioCarta database, this analysis identified 3 pathways differentially expressed at 3 months and 5 pathways differentially expressed at 1 year in Ornish participants. The KEGG pathways are:

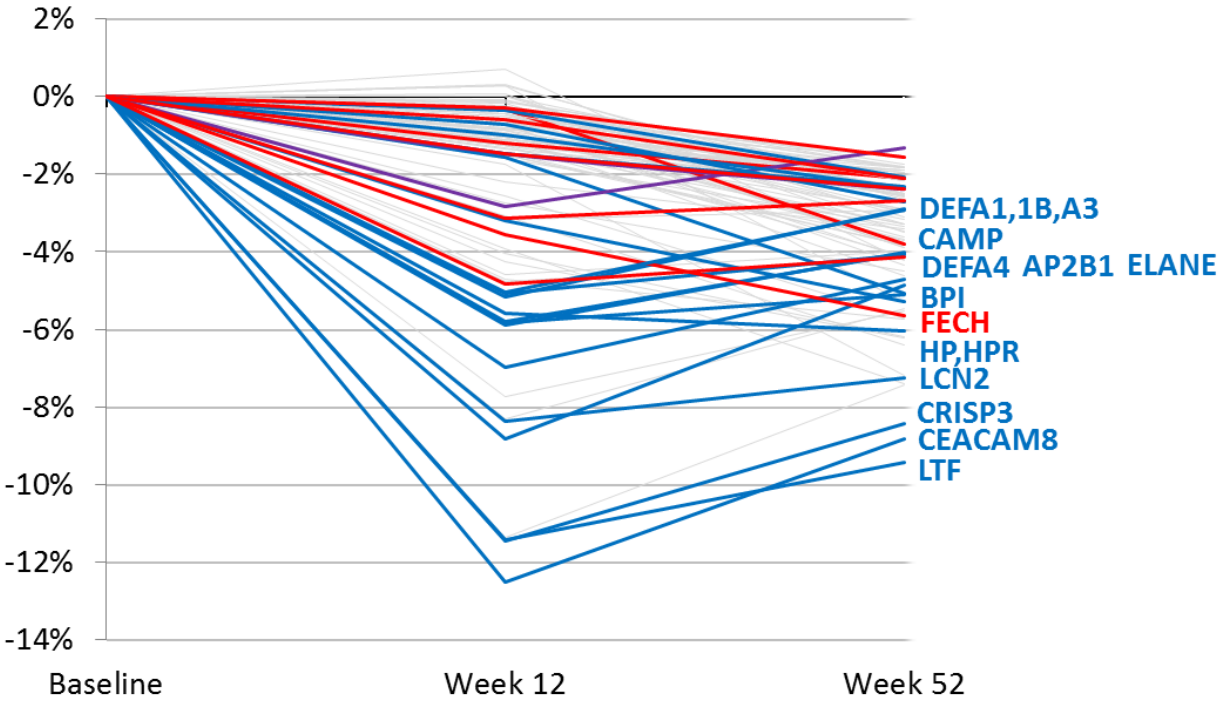
Baseline – Week 12		Genes	
hsa00640	Propanoate metabolism	26	Carboxylic acid metabolism; related to carbohydrate metabolism
hsa04912	GnRH signaling pathway	64	Synthesis/release of gonadotropins; gene expression, cell proliferation
hsa05120	Epithelial cell signaling in Hpy	53	Human diseases; Infectious diseases
Baseline – Week 52			
hsa00150	Androgen and estrogen metabolism	19	Inactivation/catabolism of androgen & estrogen in target tissues
hsa04810	Regulation of actin cytoskeleton	136	Cellular processes; Cell motility
hsa00563	GPI-anchor biosynthesis	18	Metabolism; Glycan biosynthesis/metabolism

Manuscript *in preparation*:
-Ellsworth DL, Croft DT Jr, Weyandt J, Field LA, Patney HL, Burke A, Haberkorn MJ, McDyer FA, Jellema GL, van Laar R, Mamula KA, Vernalis MN. Intensive cardiovascular risk reduction induces sustainable changes in peripheral blood gene expression. (In preparation).

Additional analysis showed that changes in gene expression mirrored changes in many CVD risk factors (Fig. 1) – dramatic decrease during the first 12 weeks, then regression toward baseline from week 13 to 52 (Fig. 2). Most cholesterol and lipid homeostasis genes showed a continual decrease in expression throughout the program similar to body weight (Fig. 2). Medication use clearly did not affect gene expression, thus expression changes may be attributed to the lifestyle change program (Table 2).

Abstract: *poster presented* (See Appendix A)*:*
- Ellsworth DL, Croft DT Jr, Burke A, Haberkorn MJ, Patney HL, Mamula KA, Vernalis MN. The importance of weight loss for effecting molecular change during intensive cardiovascular risk reduction. Obesity 2012: 30th Annual Scientific Meeting, 20-24 Sep12, San Antonio, TX.





Figures 1 and 2. Change in traditional CVD risk factors (Fig. 1) and changes in gene expression (Fig. 2) during intensive lifestyle change.

Table 2. Effects of medications on gene expression from Baseline to Week 52

Probe ID	Symbol	Fold Change All Participants (n=63)	Fold Change Lipid Lowering Medications* (n=51)	Fold Change All Medications† (n=34)
202018_s_at	LTF	–1.67	–1.67	–1.70
221748_s_at	TNS1	–1.55	–1.51	–1.43
212531_at	LCN2	–1.47	–1.44	–1.48
206676_at	CEACAM8	–1.44	–1.48	–1.68
214407_x_at	GYPB	–1.41	–1.34	–1.26
206698_at	XK	–1.41	–1.43	–1.36
206665_s_at	BCL2L1	–1.39	–1.35	–1.31
203502_at	BPGM	–1.37	–1.40	–1.41
203115_at	FECH	–1.35	–1.31	–1.28
207802_at	CRISP3	–1.32	–1.32	–1.43
208470_s_at	HP/HPR	–1.30	–1.31	–1.24
212768_s_at	OLFM4	–1.29	–1.20	–1.23
213446_s_at	IQGAP1	–1.28	–1.25	–1.22
208632_at	RNF10	–1.28	–1.25	–1.18
221627_at	TRIM10	–1.28	–1.23	–1.21
218418_s_at	KANK2	–1.28	–1.22	–1.21
217878_s_at	CDC27	–1.27	–1.26	–1.22
210244_at	CAMP	–1.27	–1.26	–1.27
200615_s_at	AP2B1	–1.26	–1.24	–1.22
205557_at	BPI	–1.25	–1.22	–1.29
211993_at	WNK1	–1.25	–1.23	–1.17

Plasma Metabolites – Collaboration with Dr. Dean Jones and Dr. Quinlyn Soltow at Emory University to profile plasma metabolites associated with CVD development continuing. We have analyzed 17 Ornish patients and 17 matched controls (all at three time points) by liquid chromatography-Fourier transform mass spectrometry (LC-FTMS). All assays run in duplicate, manuscript in preparation.

Structural and Functional Measures of Cardiovascular Health

Specific endpoints measured include ejection fraction and wall motion, coronary artery calcification scores, left and right ventricular volumes, myocardial mass, stenosis sizing and vessel diameter, plaque density and differentiation of calcified versus non-calcified plaque, and

tissue perfusion and viability. Work continues on the quantification and interpretation of the huge volumes of imaging data we have acquired.

Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Previous Subjects in the Dr. Dean Ornish Program for Reversing Heart Disease.

Methodology

The primary objective of this sub-study is to examine associations between DNA variation (in the form of 500,000+ single nucleotide polymorphisms) and participant response to the program. We are examining the influence of innate genetic variation on overall response, quantified as the risk of future cardiac events (Framingham risk), as well as response of specific cardiovascular disease risk factors. The main hypothesis is that innate variation in genes associated with lipid metabolism, protein biosynthesis, protein modification, transcription regulation and/or cell surface receptors (or other genes) will correlate positively with response to intensive lifestyle changes involving diet, exercise, meditation, yoga and group support, which may lead to improved CHD risk factor profiles and genetic markers of coronary artery disease reversal or stabilization. Participants in this study are being recruited from previous cohorts of the Dr. Dean Ornish Program for Reversing Heart Disease at Windber Medical Center (prior to implementation of the primary Molecular Profiling Protocol described above).

Status:

Enrollment in the sub-study was closed as of July 27, 2007. Data analysis is ongoing. Profiled 39 SNPs defined in recent genome-wide association studies to have an impact on CVD development or associated risk factors; influence of 23 SNPs on triglyceride response has been evaluated; data analysis is ongoing. The following manuscript is nearly complete:

- Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. SNPs associated with plasma triglyceride levels influence response during intensive cardiovascular risk reduction. (in preparation).

Four additional SNPs were genotyped, 3 in close proximity to rs17145738 and one near rs3846662. rs17145738 and rs3846662 showed significant differences between genotypes in triglyceride response in previous analyses. Of the 4 new SNPs, 2 SNPs (rs12916 and rs714052) also showed a significant difference in triglyceride response to the Ornish program between genotypes. The following **abstract *was accepted*** for poster presentation at the ASHG Meeting in San Francisco **(See Appendix A)**:

- Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. SNPs associated with plasma triglyceride levels influence response during intensive cardiovascular risk reduction. American Society of Human Genetics Annual Meeting, 6-10 Nov 12, San Francisco, CA.

Dietary Factors in the Ornish and CRC Programs – Brianne Seitz our summer intern compared dietary outcomes of CRC participants to the Ornish program and controls. Ornish participants showed improvement in most dietary factors, but there was little change in controls. Ornish participants significantly lowered their daily fat intake by more than 60% ($P<0.001$ versus controls), while increasing carbohydrate intake by 30% ($P<0.001$ versus controls). Due to the stringency of the program, initial changes among Ornish participants were larger than those in CRC participants, but there was evidence that CRC participants showed less regression over time.

Task #2: Continue the "Cardiovascular Risk Assessment and Prevention Program through the Cardiovascular Risk Clinic (CRC) at ICHP-WRI".

Methodology

This program, now called the Cardiovascular Risk Clinic, is being established as a platform to address the unique needs of retired military beneficiaries at risk for CV disease. The program mirrors the Cardiac Prevention Program (CPP) designed and established by the ICHP at WRAMC. It includes conventional and novel CV risk profiling and tailored, personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a nurse case manager, psychologist, nurse practitioners, dietitians, stress management instructors, and exercise physiologists. Validated tools to screen for and measure CV risk, stress, sleep health, compliance with dietary recommendations and exercise are standard of care. The program is an adjunct to the best medical practices provided by their primary care provider.

Phase I of the program involves each participant undergoing a comprehensive health risk assessment that is completed by a physician, followed by a four- hour "Pearls for your Heart" workshop and participants then schedule individual appointments with each modality specialist to receive education and counseling in nutrition, exercise, stress management and mind/body health. These are monthly appointments to be completed over a 4-6 month period.

Phase II of the program begins after the completion of the healthy lifestyle intervention (Phase I). During this phase each participant will again meet with the physician. During this appointment the physician will prepare the participants for the next phase and give them strategies for maintaining success on their own. The second phase of the Program provides additional reinforcement through monthly phone calls with an integrative health coach. Participants will remain in Phase 2 for five years, during which time they will come to the center for re-assessments every six months.

Status:

Study is currently ongoing.

Subject Enrollment and Demographics:

Total subject enrollment in the CRC is 216 participants; 181 remain active (127 intervention; 89 controls); 35 drop-outs; 28 control participants have transferred to the intervention arm after one year as a control. Demographic characteristics of participants are: average age 58.9 years, 58% female, 22% veterans or the spouse of a veteran, and 20% with diagnosed coronary heart disease.

In the last quarter (July 2012- 15 Sept 2012) there were a total of 394 participant visits including periodic follow up phone calls to participants enrolled in the intervention arm of the study and 114 visits by participants enrolled in the control arm of the study.

Outcome Data

The intervention cohorts have shown change in the desired direction for virtually all of the measured coronary artery disease (CAD) risk factors over the initial 4-6 month period (Table 3A). Measures of obesity including weight and BMI declined ~3.5%. Levels of total cholesterol were reduced by ~2%, and triglycerides dropped by 13%. Dietary analysis shows marked improvement in daily total and saturated fat intake, two main dietary components that contribute to plaque formation. Systolic and diastolic blood pressure decreased by nearly 7%. Measures of carotid intimal-medial thickness (CIMT) also were significantly lower after the intervention

phase. In addition, psychometric measures also significantly decreased, specifically, depression by 30% and hostility by 15%. Similarly, sleep quality improved by 25%. This data demonstrates that lifestyle change programs may be important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease.

Results from the first long-term follow up time point (6 months after completion of the intervention) are shown in Table 3B. Over the course of approximately 8-10 months, weight, BMI, total cholesterol, triglycerides, blood pressure, and CIMT measurements all maintained statistical significance proving that the positive improvements in these traditional risk factors for CAD can be maintained over a longer period of time. In addition, at this time point, depression and hostility remained significantly improved as well sleep quality.

In Table 3C, results 1 year after completion of the intervention are shown. Weight, BMI, total cholesterol, LDL cholesterol, CIMT measurements as well as psychosocial and sleep factors continue to maintain statistical significance. Most variables continue to trend in the desirable direction.

Tables 3D and 3E show the furthest time points reached thus far, 18 months and 2 years respectively after completion of the intervention. Although a relatively small sample size risk factors continue to show positive improvements.

Table 3A. Comparison of "Baseline" to "Intervention Complete" (4-6 months) data for participants in the intervention arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Intervention Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	91	194.98 (45.6)	190.48 (42.8)	-4.5	<0.00001
Body Mass Index	88	30.92 (6.1)	30.16 (6.0)	-0.8	<0.00001
Total Cholesterol (mg/dl)	85	187.59 (39.4)	184.28 (37.5)	-3.3	0.2845
High Density Lipids (mg/dl)	85	48.42 (12.6)	48.74 (11.1)	0.3	0.7003
Low Density Lipids (mg/dl)	85	112.49 (32.0)	111.98 (31.4)	-0.5	0.8370
Triglycerides (mg/dl)	85	135.40 (72.3)	117.69 (51.0)	-17.7	<0.001
Systolic Blood Pressure	85	131.48 (17.9)	125.06 (17.0)	-6.4	<0.0001
Diastolic Blood Pressure	85	80.64 (11.1)	75.48 (9.2)	-5.2	<0.00001
Depression Scale [CES-D]	85	10.32 (9.3)	7.11 (6.9)	-3.2	<0.00001
Hostility Scale [Cook-Medley]	85	7.26 (4.7)	6.20 (4.2)	-1.1	<0.001
Perceived Stress Scale [PSS]	85	13.09 (5.9)	10.73 (5.4)	-2.4	<0.0001
Daily Total Fat (grams)	79	64.05 (32.6)	52.43 (22.6)	-11.6	<0.01
Daily Saturated Fat (grams)	79	19.85 (10.7)	16.38 (8.8)	-3.5	<0.01
Avg. CCA/Mean IMT	85	0.748 (0.1608)	0.700 (0.1407)	-0.048	<0.00001
Avg. CCA / Max IMT	85	0.862 (0.1856)	0.805 (0.1506)	-0.1	<0.00001
Fasting Glucose (mg/dl)	85	104 (33.6)	101 (24.4)	-3.0	0.2996
HgbA1c	85	6.0 (1.07)	6.0 (1.10)	-0.1	0.2433
Cortisol	84	11.9 (3.93)	13.7 (3.98)	1.8	<0.001
TSH	85	1.94 (1.063)	2.14 (1.305)	0.2	0.1285
Epworth Sleepiness Scale (0 to 24)	84	8 (4.5)	7 (4.2)	-0.9	<0.01
Pittsburgh Sleep Quality Index (0-21)	83	8 (4.3)	6 (3.9)	-1.3	<0.0001

Table 3B. Change in outcome variables 6 months after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average 10 month value (SD)	Average Change	P-Value
Weight (lbs.)	60	190.84 (45.1)	184.07 (41.2)	-6.8	<0.00001
Body Mass Index	60	30.47 (6.0)	29.59 (5.7)	-0.9	<0.001
Total Cholesterol (mg/dl)	61	189.41 (42.4)	178.25 (38.1)	-11.2	<0.05
High Density Lipids (mg/dl)	61	49.28 (13.7)	48.44 (12.8)	-0.8	0.3427
Low Density Lipids (mg/dl)	61	112.38 (32.8)	105.56 (31.5)	-6.8	0.0896
Triglycerides (mg/dl)	61	141.64 (72.7)	122.25 (65.6)	-19.4	<0.01
Systolic Blood Pressure	60	131.03 (18.0)	126.40 (17.3)	-4.6	<0.05
Diastolic Blood Pressure	60	80.50 (11.0)	74.97 (9.1)	-5.5	<0.001
Depression Scale [CES-D]	59	9.37 (9.3)	7.12 (9.1)	-2.3	<0.05
Hostility Scale [Cook-Medley]	59	7.00 (4.6)	6.56 (4.3)	-0.4	0.2956
Perceived Stress Scale [PSS]	59	12.47 (6.1)	10.44 (6.6)	-2.0	<0.01
Daily Total Fat (grams)	55	64.33 (33.0)	51.42 (20.8)	-12.9	<0.05
Daily Saturated Fat (grams)	55	19.56 (10.6)	15.79 (8.1)	-3.8	<0.05
Avg. CCA/Mean IMT	60	0.772 (0.1446)	0.699 (0.1407)	-0.073	<0.00001
Avg. CCA / Max IMT	60	0.891 (0.1680)	0.798 (0.1508)	-0.1	<0.00001
Fasting Glucose (mg/dl)	62	102 (20.0)	102 (25.5)	0.5	0.8710
HgbA1c	61	6.1 (0.94)	6.0 (1.19)	-0.1	0.1140
Cortisol	59	11.8 (3.96)	13.6 (4.02)	1.8	<0.01
TSH	61	1.99 (1.180)	2.31 (1.527)	0.3	<0.05
Epworth Sleepiness Scale (0 to 24)	57	8 (4.4)	7 (4.3)	-1.1	<0.01
Pittsburgh Sleep Quality Index (0-21)	57	7 (4.0)	6 (3.6)	-1.4	<0.001

Table 3C. Change in outcome variables 1 year after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average 10 month value (SD)	Average Change	P-Value
Weight (lbs.)	27	191.01 (41.7)	183.30 (38.3)	-7.7	<0.0001
Body Mass Index	26	30.47 (5.7)	29.24 (5.1)	-1.2	<0.001
Total Cholesterol (mg/dl)	26	187.96 (41.0)	171.38 (37.9)	-16.6	<0.01
High Density Lipids (mg/dl)	26	52.50 (15.6)	50.46 (13.2)	-2.0	0.2047
Low Density Lipids (mg/dl)	26	109.81 (30.8)	98.65 (27.6)	-11.2	<0.01
Triglycerides (mg/dl)	26	131.12 (70.1)	111.81 (54.6)	-19.3	0.0563
Systolic Blood Pressure	26	132.15 (14.0)	125.85 (14.1)	-6.3	0.0591
Diastolic Blood Pressure	26	79.54 (10.2)	75.54 (7.8)	-4.0	0.0686
Depression Scale [CES-D]	26	10.00 (9.7)	6.92 (7.4)	-3.1	<0.05
Hostility Scale [Cook-Medley]	26	7.96 (4.5)	6.69 (4.2)	-1.3	<0.05
Perceived Stress Scale [PSS]	26	12.77 (7.1)	10.23 (6.3)	-2.5	<0.01
Daily Total Fat (grams)	26	69.24 (32.2)	45.08 (13.8)	-24.2	<0.001
Daily Saturated Fat (grams)	26	20.91 (10.9)	13.31 (4.8)	-7.6	<0.01
Avg. CCA/Mean IMT	26	0.860 (0.1249)	0.719 (0.1172)	-0.141	<0.00001
Avg. CCA / Max IMT	26	0.996 (0.1510)	0.828 (0.1365)	-0.2	<0.00001

Fasting Glucose (mg/dl)	26	101 (13.7)	104 (22.3)	2.7	0.4426
HgbA1c	26	6.2 (1.05)	6.0 (1.21)	-0.2	<0.01
Cortisol	26	12.1 (4.57)	13.9 (3.07)	1.8	<0.05
TSH	26	2.34 (1.387)	2.39 (1.371)	0.0	0.8242
Epworth Sleepiness Scale (0 to 24)	25	8 (4.3)	7 (3.6)	-1.1	0.1034
Pittsburgh Sleep Quality Index (0-21)	25	9 (5.0)	6 (3.2)	-2.2	<0.01

Table 3D. Change in outcome variables 18 months after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average 10 month value (SD)	Average Change	P-Value
Weight (lbs.)	14	188.96 (42.7)	182.93 (38.0)	-6.0	<0.05
Body Mass Index	14	29.56 (4.2)	28.44 (3.8)	-1.1	<0.01
Total Cholesterol (mg/dl)	14	179.14 (46.2)	175.29 (43.6)	-3.9	0.6836
High Density Lipids (mg/dl)	14	52.36 (10.8)	49.21 (7.2)	-3.1	0.2554
Low Density Lipids (mg/dl)	14	105.50 (35.6)	105.71 (32.6)	0.2	0.9780
Triglycerides (mg/dl)	14	112.71 (52.7)	101.00 (41.5)	-11.7	0.2346
Systolic Blood Pressure	14	128.29 (14.5)	129.86 (20.1)	1.6	0.7470
Diastolic Blood Pressure	14	77.86 (11.9)	74.00 (8.5)	-3.9	0.1917
Depression Scale [CES-D]	14	11.00 (11.2)	10.36 (10.7)	-0.6	0.7789
Hostility Scale [Cook-Medley]	14	7.14 (4.6)	6.57 (4.2)	-0.6	0.5616
Perceived Stress Scale [PSS]	14	12.21 (8.1)	12.36 (8.7)	0.1	0.9482
Daily Total Fat (grams)	14	58.69 (30.7)	63.48 (52.2)	4.8	0.7661
Daily Saturated Fat (grams)	14	18.75 (12.2)	24.69 (27.5)	5.9	0.4872
Avg. CCA/Mean IMT	13	0.853 (0.1176)	0.698 (0.1431)	-0.156	<0.00001
Avg. CCA / Max IMT	13	0.982 (0.1184)	0.809 (0.1548)	-0.2	<0.00001
Fasting Glucose (mg/dl)	14	101 (12.3)	103 (33.7)	2.4	0.7658
HgbA1c	14	6.3 (1.16)	6.1 (1.21)	-0.2	<0.01
Cortisol	14	13.0 (4.66)	13.2 (4.60)	0.2	0.9239
TSH	14	2.18 (1.366)	2.24 (1.724)	0.1	0.8444
Epworth Sleepiness Scale (0 to 24)	14	7 (4.6)	6 (4.4)	-1.0	0.2544
Pittsburgh Sleep Quality Index (0-21)	14	8 (4.9)	6 (3.0)	-2.1	<0.05

Table 3E. Change in outcome variables 2 years after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average 10 month value (SD)	Average Change	P-Value
Weight (lbs.)	14	188.96 (42.7)	183.63 (39.7)	-5.3	<0.05
Body Mass Index	14	29.56 (4.2)	28.71 (4.0)	-0.8	<0.05
Total Cholesterol (mg/dl)	14	179.14 (46.2)	174.64 (48.2)	-4.5	0.7302
High Density Lipids (mg/dl)	14	52.36 (10.8)	50.21 (8.0)	-2.1	0.3804
Low Density Lipids (mg/dl)	14	105.50 (35.6)	106.57 (37.8)	1.1	0.9158
Triglycerides (mg/dl)	14	112.71 (52.7)	89.86 (51.3)	-22.9	0.0637
Systolic Blood Pressure	14	128.29 (14.5)	126.00 (16.2)	-2.3	0.6571
Diastolic Blood Pressure	14	77.86 (11.9)	73.29 (8.6)	-4.6	0.1246

Depression Scale [CES-D]	14	11.00 (11.2)	7.93 (9.8)	-3.1	0.0673
Hostility Scale [Cook-Medley]	14	7.14 (4.6)	6.07 (4.3)	-1.1	0.1969
Perceived Stress Scale [PSS]	14	12.21 (8.1)	9.43 (7.2)	-2.8	0.0565
Daily Total Fat (grams)	14	58.69 (30.7)	63.44 (18.8)	4.8	0.6025
Daily Saturated Fat (grams)	14	18.75 (12.2)	19.69 (8.1)	0.9	0.8048
Avg. CCA/Mean IMT	14	0.846 (0.1166)	0.650 (0.1460)	-0.196	<0.00001
Avg. CCA / Max IMT	14	0.974 (0.1180)	0.750 (0.1617)	-0.2	<0.00001
Fasting Glucose (mg/dl)	14	101 (12.3)	105 (31.5)	4.1	0.5713
HgbA1c	14	6.3 (1.16)	5.7 (1.07)	-0.6	<0.001
Cortisol	14	13.0 (4.66)	13.8 (2.98)	0.7	0.5582
TSH	14	2.18 (1.366)	2.83 (2.280)	0.6	0.0592
Epworth Sleepiness Scale (0 to 24)	14	7 (4.6)	5 (3.5)	-1.9	<0.05
Pittsburgh Sleep Quality Index (0-21)	14	8 (4.9)	6 (3.7)	-2.1	<0.05

In subjects randomized to the control arm of the study, who do not participate in the lifestyle change intervention showed no significant changes in risk factors, except for CIMT and cortisol at the first 6 month time point (Table 4A). Subsequent follow up time points (Table 4B: 6 month time point; Table 4C: 1 year; Table 4D: 18 months; and Table 4E: 2 years) continue to show that most risk factors did not improve and those risk factors that did not maintain improvement at the next time point, perhaps this improvement could be attributed to small sample size and large variability among the participant's results. This lack of consistent improvement within the control arm further proves the benefits of a team-base, patient-centered lifestyle change model in improving risk for developing heart disease.

Table 4A. Change in outcome variables from baseline to "waiting period complete" period for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	67	189.08 (43.0)	188.63 (42.5)	-0.4	0.6694
Body Mass Index	67	30.97 (6.4)	30.84 (6.2)	-0.1	0.6791
Total Cholesterol (mg/dl)	68	190.87 (37.0)	186.60 (35.9)	-4.3	0.2413
High Density Lipids (mg/dl)	68	49.85 (14.5)	49.07 (12.3)	-0.8	0.3747
Low Density Lipids (mg/dl)	68	115.50 (31.2)	110.56 (31.0)	-4.9	0.1406
Triglycerides (mg/dl)	68	130.40 (65.5)	133.00 (68.5)	2.6	0.6591
Systolic Blood Pressure	68	129.85 (17.8)	130.59 (21.0)	0.7	0.7205
Diastolic Blood Pressure	68	79.06 (10.2)	77.85 (10.2)	-1.2	0.2927
Depression Scale [CES-D]	67	11.61 (10.2)	10.61 (8.9)	-1.0	0.2772
Hostility Scale [Cook-Medley]	67	7.60 (4.9)	7.30 (5.1)	-0.3	0.3442
Perceived Stress Scale [PSS]	67	13.33 (7.1)	13.10 (7.7)	-0.2	0.7121
Daily Total Fat (grams)	57	72.24 (32.3)	68.37 (32.7)	-3.9	0.4075
Daily Saturated Fat (grams)	57	22.25 (10.2)	21.14 (11.2)	-1.1	0.4542
Avg. CCA / Mean IMT	67	0.801 (0.2040)	0.746 (0.1778)	-0.055	<0.001
Avg. CCA / Max IMT	67	0.924 (0.2406)	0.850 (0.1990)	-0.1	<0.0001
Fasting Glucose (mg/dl)	68	108 (37.4)	109 (41.2)	0.3	0.9243
HgbA1c	67	6.0 (1.32)	6.0 (1.10)	0.0	0.8779
Cortisol	68	12.0 (4.31)	13.6 (4.56)	1.6	<0.001

TSH	66	1.99 (1.253)	2.07 (1.088)	0.1	0.5620
Epworth Sleepiness Scale (0 to 24)	66	8 (4.3)	8 (3.9)	-0.4	0.4011
Pittsburgh Sleep Quality Index (0-21)	66	7 (3.6)	7 (3.5)	-0.2	0.5784

Table 4B. Change in outcome variables at the 6 month time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	50	194.80 (43.7)	194.68 (45.7)	-0.1	0.9370
Body Mass Index	49	31.63 (6.7)	31.41 (6.8)	-0.2	0.4642
Total Cholesterol (mg/dl)	50	194.94 (33.8)	183.84 (34.0)	-11.1	<0.01
High Density Lipids (mg/dl)	50	51.06 (15.2)	49.30 (17.6)	-1.8	0.3313
Low Density Lipids (mg/dl)	50	117.80 (27.7)	110.54 (27.8)	-7.3	<0.05
Triglycerides (mg/dl)	50	133.98 (65.0)	128.32 (57.8)	-5.7	0.4946
Systolic Blood Pressure	50	130.48 (16.2)	131.00 (24.8)	0.5	0.8496
Diastolic Blood Pressure	50	79.16 (10.4)	77.76 (11.8)	-1.4	0.4013
Depression Scale [CES-D]	48	10.98 (9.8)	11.02 (9.9)	0.0	0.9692
Hostility Scale [Cook-Medley]	48	7.40 (5.2)	7.06 (4.6)	-0.3	0.3528
Perceived Stress Scale [PSS]	48	12.48 (7.2)	12.21 (7.0)	-0.3	0.7484
Daily Total Fat (grams)	47	71.80 (34.2)	65.51 (28.4)	-6.3	0.2617
Daily Saturated Fat (grams)	47	22.03 (10.8)	22.19 (10.5)	0.2	0.9265
Avg. CCA / Mean IMT	48	0.847 (0.1892)	0.740 (0.1719)	-0.107	<0.00001
Avg. CCA / Max IMT	48	0.976 (0.2225)	0.855 (0.1923)	-0.1	<0.0001
Fasting Glucose (mg/dl)	50	115 (41.5)	114 (41.6)	-0.6	0.8686
HgbA1c	50	6.2 (1.43)	6.2 (1.19)	-0.1	0.4052
Cortisol	50	12.0 (4.08)	13.1 (3.98)	1.1	0.0686
TSH	50	1.96 (1.267)	2.12 (0.935)	0.2	0.3041
Epworth Sleepiness Scale (0 to 24)	48	8 (4.2)	8 (3.9)	-0.1	0.8213
Pittsburgh Sleep Quality Index (0-21)	48	6 (3.5)	6 (3.4)	0.1	0.7277

Table 4C. Change in outcome variables at year 1 time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	22	199.55 (41.6)	194.65 (42.3)	-4.9	<0.05
Body Mass Index	22	31.75 (5.9)	30.68 (6.1)	-1.1	<0.05
Total Cholesterol (mg/dl)	21	196.86 (39.3)	182.33 (37.8)	-14.5	<0.05
High Density Lipids (mg/dl)	21	54.38 (19.0)	49.86 (17.0)	-4.5	<0.01
Low Density Lipids (mg/dl)	21	116.43 (32.6)	107.43 (30.9)	-9.0	0.2162
Triglycerides (mg/dl)	21	138.71 (81.7)	124.76 (77.1)	-14.0	0.1953
Systolic Blood Pressure	21	131.24 (14.8)	129.62 (19.6)	-1.6	0.6637

Diastolic Blood Pressure	21	80.76 (9.8)	77.81 (12.5)	-3.0	0.1814
Depression Scale [CES-D]	21	9.48 (9.4)	9.43 (10.4)	0.0	0.9682
Hostility Scale [Cook-Medley]	21	7.76 (5.5)	7.95 (4.7)	0.2	0.7558
Perceived Stress Scale [PSS]	21	11.52 (8.0)	12.24 (6.6)	0.7	0.5529
Daily Total Fat (grams)	16	80.18 (33.7)	67.22 (35.2)	-13.0	0.1789
Daily Saturated Fat (grams)	16	24.46 (10.0)	21.61 (10.9)	-2.8	0.3257
Avg. CCA / Mean IMT	21	0.914 (0.1655)	0.774 (0.1364)	-0.141	<0.00001
Avg. CCA / Max IMT	21	1.057 (0.1763)	0.882 (0.1520)	-0.2	<0.00001
Fasting Glucose (mg/dl)	21	121 (46.6)	111 (47.1)	-9.9	0.1832
HgbA1c	21	6.4 (1.46)	6.1 (1.00)	-0.4	0.0886
Cortisol	21	13.7 (4.34)	13.3 (4.50)	-0.4	0.7584
TSH	21	1.84 (1.132)	2.03 (0.822)	0.2	0.2164
Epworth Sleepiness Scale (0 to 24)	21	9 (4.7)	7 (4.1)	-1.1	0.1530
Pittsburgh Sleep Quality Index (0-21)	21	7 (4.1)	6 (3.6)	-0.2	0.6863

Table 4D. Change in outcome variables 18 month time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	13	204.22 (40.8)	198.86 (47.3)	-5.4	0.3780
Body Mass Index	13	33.15 (6.0)	32.19 (7.0)	-1.0	0.3696
Total Cholesterol (mg/dl)	13	211.00 (35.7)	201.31 (40.9)	-9.7	0.4169
High Density Lipids (mg/dl)	13	56.77 (21.2)	54.92 (20.4)	-1.8	0.4082
Low Density Lipids (mg/dl)	13	129.00 (31.0)	120.69 (35.6)	-8.3	0.5152
Triglycerides (mg/dl)	13	136.08 (67.7)	127.92 (98.0)	-8.2	0.6172
Systolic Blood Pressure	9	134.89 (17.0)	127.33 (24.5)	-7.6	0.3164
Diastolic Blood Pressure	9	81.33 (10.5)	79.78 (9.6)	-1.6	0.6560
Depression Scale [CES-D]	12	8.00 (5.0)	9.00 (7.6)	1.0	0.5124
Hostility Scale [Cook-Medley]	12	6.92 (5.1)	7.00 (5.2)	0.1	0.9030
Perceived Stress Scale [PSS]	12	10.58 (5.6)	11.75 (6.3)	1.2	0.5632
Daily Total Fat (grams)	10	72.35 (27.3)	80.50 (31.1)	8.1	0.4831
Daily Saturated Fat (grams)	10	21.82 (6.9)	25.60 (8.5)	3.8	0.2724
Avg. CCA / Mean IMT	13	0.935 (0.1549)	0.751 (0.1475)	-0.183	<0.0001
Avg. CCA / Max IMT	13	1.069 (0.1786)	0.862 (0.1681)	-0.2	<0.0001
Fasting Glucose (mg/dl)	13	113 (38.6)	104 (21.8)	-9.2	0.2277
HgbA1c	13	6.4 (1.31)	5.9 (0.69)	-0.5	0.0673
Cortisol	13	13.3 (4.52)	11.4 (4.93)	-1.9	0.2866
TSH	13	1.65 (0.836)	1.92 (0.564)	0.3	0.1088
Epworth Sleepiness Scale (0 to 24)	12	9 (4.8)	7 (4.0)	-1.4	0.1857
Pittsburgh Sleep Quality Index (0-21)	12	7 (4.7)	7 (4.3)	-0.5	0.6595

Table 4E. Change in outcome variables at Year 2 time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	8	192.63 (43.1)	186.48 (38.6)	-6.2	<0.05
Body Mass Index	9	32.43 (5.9)	30.80 (5.3)	-1.6	0.0917
Total Cholesterol (mg/dl)	8	209.50 (36.3)	182.75 (48.2)	-26.8	0.1127
High Density Lipids (mg/dl)	8	62.75 (25.4)	56.88 (22.9)	-5.9	0.1441
Low Density Lipids (mg/dl)	8	122.38 (25.2)	99.50 (35.3)	-22.9	0.2084
Triglycerides (mg/dl)	8	137.50 (82.4)	131.75 (64.5)	-5.8	0.7400
Systolic Blood Pressure	8	135.50 (17.3)	135.50 (25.8)	0.0	1.0000
Diastolic Blood Pressure	8	82.75 (10.3)	73.13 (10.3)	-9.6	<0.01
Depression Scale [CES-D]	6	5.00 (3.2)	4.00 (3.6)	-1.0	0.4466
Hostility Scale [Cook-Medley]	6	7.50 (4.1)	9.00 (4.4)	1.5	0.2264
Perceived Stress Scale [PSS]	6	9.50 (7.0)	8.50 (6.4)	-1.0	0.6884
Daily Total Fat (grams)	3	58.89 (19.6)	79.45 (16.9)	20.6	0.0850
Daily Saturated Fat (grams)	3	17.28 (6.3)	29.19 (11.2)	11.9	0.1616
Avg. CCA / Mean IMT	8	0.915 (0.1560)	0.694 (0.1393)	-0.221	<0.001
Avg. CCA / Max IMT	8	1.037 (0.1771)	0.775 (0.1518)	-0.3	<0.001
Fasting Glucose (mg/dl)	8	121 (45.9)	107 (20.2)	-14.3	0.2917
HgbA1c	8	6.4 (1.27)	5.8 (0.65)	-0.7	0.0740
Cortisol	8	12.8 (5.25)	12.4 (3.65)	-0.5	0.8034
TSH	8	1.61 (0.804)	2.54 (1.447)	0.9	0.2154
Epworth Sleepiness Scale (0 to 24)	6	9 (4.0)	8 (4.2)	-1.2	0.3522
Pittsburgh Sleep Quality Index (0-21)	6	5 (2.1)	6 (3.4)	0.3	0.7497

Adverse Events

All adverse events are submitted to and adjudicated by the Windber Medical Center Institutional Review Board and TATRC after review by both the Principal Investigator and Medical Monitor. There were no adverse events in either arm of the study during the last quarter. To date there have been a total of 16 adverse events, 8 in the intervention and 8 in the control arm of the study, all deemed serious events, not related and not expected. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Therefore, all 16 events were considered serious due to inpatient hospitalizations. There were 7 non-cardiac and 1 cardiac adverse events in the intervention arm of the study. No deaths occurred and none of these adverse events were deemed to be study related. There were 5 non-cardiac and 3 cardiac adverse events in the control arm of the study. No deaths occurred and none of these adverse events were deemed to be study related.

NMR Lipid Panel and Biomarkers

This year for the CRC program, approximately 5,570 aliquots have been made summarized by the following:

PAXGene Tubes	296
RBCs	586

Plasma samples		Serum samples	
NMR lipids	293	Adiponectin	298
Leptin	293	Serum amyloid A	298
CRP	293	Vitamin D	298
Resistin	293	Lp(a)	298
Insulin	293	Extra Serum	1165
Extra plasma	866		

SubTask #2.1: Continue "Stress Therapy Empowering Prevention (STEP)" component to the CRC.

Methodology

This is a collaborative study involving researchers from WRI and WRNMMC and is modeled after the Caretakers Optimizing Readiness through Preventive Strategies (CORPS), designed by the ICHP at WRNMMC, except that it targets participants with chronic disease. The purpose of this task is to determine the degree of stress, sleep disturbance, and cardiovascular disease risk in patients who have been diagnosed with breast cancer or are at high risk of developing breast disease.

In the first part of the intervention, patients will be randomized to a 12 week Healthy Lifestyle intervention group or a non-intervention group. During this phase, each intervention participant undergo a comprehensive health risk assessment that is completed by a physician, followed by mandatory attendance to on-site group sessions in which they will participate in 1 hour of stress management, 30 minutes of nutrition education every week, and 30 minutes of exercise alternated with 30 minutes of mind/body health every other week. In addition, the nurse will provide educational lectures on various health topics during 4 sessions. After completing Phase I, patients will participate in a five year healthy lifestyle intervention or control group.

During phase II each intervention participant will again meet with the physician. During this appointment the physician will prepare the participants for the next phase and give them strategies for maintaining success on their own. The second phase of the program provides additional reinforcement through monthly phone calls with an integrative health coach. Participants will remain in Phase II for five years, during which time they will come to the center for re-assessments every six months.

We hypothesize that the 12 week healthy lifestyle interventions will significantly reduce stress, sleep disturbances, and cardiovascular risk in patients at risk for, or already diagnosed with, breast cancer.

Status:
Study is currently being closed for enrollment but will remain open for data analysis. The following **manuscript *was published*** **(See Appendix A)**:

-Burke A, Ellsworth DL, Vernalis MN. Stress Therapy Empowers Prevention (STEP): A Healthy-Lifestyle Program for Breast Cancer Patients. J Oncol Navig Surviv 2012;3(1):8-14.

Subject Enrollment and Demographics:
Total subject enrollment was 18 (intervention only); 10 active; 8 dropouts. Demographic characteristics of participants were: average age 65.6 years, 28% veterans or the spouse of a veteran, 6% have diagnosed coronary heart disease, and 61% have diagnosed breast cancer. Due to the lack of public interest we were unable to recruit a sufficient number of participants to keep this protocol open. The protocol was closed for enrollment on September 1, 2012 but will remain open for data analysis.

In the last quarter (July 2012- 15 Sept 2012) there were a total of 8 participant visits including periodic follow up phone calls made to participants.

Outcomes Data:
Overall participants showed change in the desired direction for most of the measured coronary artery disease (CAD) risk factors over the 2 years of the program (see Tables 5A-5D below). No participants were enrolled into the control arm of the study and lack of statistically significant levels of improvement in some measures may be attributable to small sample size and wide variability in some measures.

Table 5A. Comparison of baseline to Week 12 data for participants in the STEP Program

Category / Metrics	N	Average Baseline Value (SD)	Average Week 12 Value (SD)	Average Change	P Value
Weight (lbs.)	16	182.57 (35.9)	179.30 (33.0)	-3.3	<0.01
Body Mass Index	16	32.83 (6.3)	32.04 (5.9)	-0.8	<0.01
Total Cholesterol (mg/dl)	16	198.38 (36.4)	196.69 (44.2)	-1.7	0.7954
High Density Lipids (mg/dl)	16	54.44 (12.4)	52.25 (12.8)	-2.2	0.0928
Low Density Lipids (mg/dl)	16	114.50 (28.7)	118.63 (38.4)	4.1	0.5290
Triglycerides (mg/dl)	16	155.13 (90.6)	132.81 (73.4)	-22.3	0.0926
Systolic Blood Pressure	16	134.75 (18.8)	124.50 (14.1)	-10.3	0.0763
Diastolic Blood Pressure	16	80.63 (11.3)	73.75 (8.1)	-6.9	<0.05
Depression Scale [CES-D]	16	15.31 (10.2)	11.44 (10.4)	-3.9	0.0914
Hostility Scale [Cook-Medley]	16	7.06 (4.4)	5.25 (3.3)	-1.8	0.0720
Daily Total Fat (grams	8	58.62 (39.1)	44.48 (5.8)	-14.1	0.3394
Daily Saturated Fat (grams)	8	19.77 (19.5)	11.77 (3.4)	-8.0	0.2853
Perceived Stress Scale [PSS]	16	17.00 (7.2)	12.88 (6.5)	-4.1	<0.05
Avg. CCA/Mean IMT	16	0.735 (0.1488)	0.810 (0.1677)	0.075	<0.01
Avg. CCA / Max IMT	16	0.865 (0.1556)	0.928 (0.2046)	0.1	<0.05
Fasting Glucose (mg/dl)	16	107 (28.8)	109 (25.7)	2.4	0.6604
HgbA1c	16	6.3 (0.87)	6.5 (0.77)	0.2	0.3545
Cortisol	16	12.8 (3.83)	16.5 (5.44)	3.7	0.0507
TSH	16	1.71 (1.342)	2.07 (1.674)	0.4	0.2887
Epworth Sleepiness Scale (0 to 24)	16	9 (4.5)	8 (4.2)	-0.9	0.4320
Pittsburgh Sleep Quality Index (0-21)	16	10 (4.8)	8 (4.4)	-2.5	0.0512

Table 5B. Comparison of baseline to Year 1 data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	14	180.49 (35.5)	177.30 (32.7)	-3.2	<0.05
Body Mass Index	14	32.49 (6.4)	31.66 (6.0)	-0.8	<0.05
Total Cholesterol (mg/dl)	14	201.07 (37.3)	200.21 (45.8)	-0.9	0.9083
High Density Lipids (mg/dl)	14	54.64 (13.1)	52.14 (13.7)	-2.5	0.0715
Low Density Lipids (mg/dl)	14	116.79 (30.0)	121.57 (40.2)	4.8	0.5252
Triglycerides (mg/dl)	14	157.21 (95.4)	136.71 (76.1)	-20.5	0.1721
Systolic Blood Pressure	14	134.00 (18.4)	125.14 (15.0)	-8.9	0.1451
Diastolic Blood Pressure	14	79.57 (11.3)	72.86 (8.3)	-6.7	0.0838
Depression Scale [CES-D]	14	13.71 (9.7)	11.29 (11.0)	-2.4	0.1056
Hostility Scale [Cook-Medley]	14	6.36 (4.2)	4.79 (3.2)	-1.6	0.3330
Perceived Stress Scale [PSS]	14	16.29 (7.4)	12.71 (6.8)	-3.6	<0.05
Daily Total Fat (grams)	10	61.64 (36.5)	54.64 (25.4)	-7.0	0.4039
Daily Saturated Fat (grams)	10	21.49 (18.2)	15.26 (8.5)	-6.2	0.1507
Avg. CCA/Mean IMT	14	0.745 (0.1557)	0.826 (0.1718)	0.081	<0.01
Avg. CCA / Max IMT	14	0.879 (0.1607)	0.948 (0.2110)	0.1	<0.05
Fasting Glucose (mg/dl)	14	109 (30.6)	111 (27.4)	2.0	0.7535
HgbA1c	14	6.4 (0.91)	6.6 (0.77)	0.2	0.3356
Cortisol	14	12.4 (3.61)	16.9 (5.61)	4.5	<0.05
TSH	14	1.66 (1.419)	2.19 (1.766)	0.5	0.1559
Epworth Sleepiness Scale (0 to 24)	14	8 (4.5)	8 (4.5)	-0.6	0.6020
Pittsburgh Sleep Quality Index (0-21)	14	10 (5.1)	8 (4.6)	-2.7	0.0639

Table 5C. Comparison of baseline to 18 month data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	10	189.23 (36.5)	188.36 (33.3)	-0.9	0.7195
Body Mass Index	10	33.66 (7.0)	33.37 (6.2)	-0.3	0.4956
Total Cholesterol (mg/dl)	10	200.50 (40.4)	211.50 (60.3)	11.0	0.4066
High Density Lipids (mg/dl)	10	53.50 (13.3)	52.00 (13.2)	-1.5	0.3974
Low Density Lipids (mg/dl)	10	113.40 (31.1)	126.60 (43.3)	13.2	0.1605
Triglycerides (mg/dl)	10	180.80 (103.6)	163.20 (115.1)	-17.6	0.3961
Systolic Blood Pressure	10	135.80 (20.2)	137.40 (18.0)	1.6	0.8362
Diastolic Blood Pressure	10	79.40 (12.6)	75.20 (11.8)	-4.2	0.2921
Depression Scale [CES-D]	10	13.20 (9.6)	6.80 (7.1)	-6.4	<0.05
Hostility Scale [Cook-Medley]	10	7.40 (4.4)	5.90 (3.6)	-1.5	0.1604
Perceived Stress Scale [PSS]	10	16.00 (8.2)	8.90 (6.8)	-7.1	<0.01
Daily Total Fat (grams)	10	61.64 (36.5)	37.96 (12.0)	-23.7	0.0766
Daily Saturated Fat (grams)	10	21.49 (18.2)	10.20 (4.2)	-11.3	0.0943

Avg. CCA/Mean IMT	10	0.751 (0.1695)	0.775 (0.1719)	0.024	0.4697
Avg. CCA / Max IMT	10	0.888 (0.1815)	0.919 (0.2275)	0.0	0.4407
Fasting Glucose (mg/dl)	10	110 (34.8)	112 (39.7)	1.8	0.6648
HgbA1c	10	6.4 (0.98)	6.7 (1.52)	0.3	0.3126
Cortisol	10	12.8 (3.83)	13.1 (4.67)	0.3	0.8983
TSH	10	1.64 (1.577)	1.44 (1.243)	-0.2	0.5141
Epworth Sleepiness Scale (0 to 24)	10	8 (4.7)	7 (4.0)	-0.7	0.5496
Pittsburgh Sleep Quality Index (0-21)	10	10 (5.0)	7 (3.4)	-3.1	0.1121

Table 5D. Comparison of baseline to year 2 data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	10	189.23 (36.5)	184.84 (31.3)	-4.4	0.1403
Body Mass Index	10	33.66 (7.0)	32.58 (5.7)	-1.1	0.1971
Total Cholesterol (mg/dl)	10	200.50 (40.4)	194.60 (53.8)	-5.9	0.5141
High Density Lipids (mg/dl)	10	53.50 (13.3)	48.70 (11.9)	-4.8	<0.001
Low Density Lipids (mg/dl)	10	113.40 (31.1)	114.70 (43.3)	1.3	0.8637
Triglycerides (mg/dl)	10	180.80 (103.6)	162.20 (118.8)	-18.6	0.4207
Systolic Blood Pressure	10	135.80 (20.2)	132.60 (15.3)	-3.2	0.4981
Diastolic Blood Pressure	10	79.40 (12.6)	76.40 (10.6)	-3.0	0.3412
Depression Scale [CES-D]	10	13.20 (9.6)	12.00 (11.9)	-1.2	0.7045
Hostility Scale [Cook-Medley]	10	7.40 (4.4)	6.40 (5.3)	-1.0	0.3765
Perceived Stress Scale [PSS]	10	16.00 (8.2)	13.00 (9.1)	-3.0	0.2401
Daily Total Fat (grams)	10	61.64 (36.5)	56.12 (20.1)	-5.5	0.7037
Daily Saturated Fat (grams)	10	21.49 (18.2)	17.05 (6.0)	-4.4	0.5093
Avg. CCA/Mean IMT	10	0.751 (0.1695)	0.738 (0.1484)	-0.013	0.7605
Avg. CCA / Max IMT	10	0.888 (0.1815)	0.845 (0.1683)	0.0	0.3379
Fasting Glucose (mg/dl)	10	110 (34.8)	111 (30.9)	0.6	0.8474
HgbA1c	10	6.4 (0.98)	6.4 (0.94)	0.0	0.5987
Cortisol	10	12.8 (3.83)	14.7 (4.31)	1.8	0.1363
TSH	10	1.64 (1.577)	2.22 (1.635)	0.6	0.2716
Epworth Sleepiness Scale (0 to 24)	10	8 (4.7)	7 (4.5)	-0.7	0.6380
Pittsburgh Sleep Quality Index (0-21)	10	10 (5.0)	7 (4.3)	-3.1	0.0895

During this year, we received data on the following variables for CRC participants. Analysis will be forthcoming.

NMR lipid panel	296	Insulin	106
CRP	148	Adiponectin	0
Leptin	106	Serum Amyloid	0
Lipoprotein (a)	0	Resistin	106
Vitamin D	69		

Adverse Events:

All adverse events are submitted to and adjudicated by the Windber Medical Center Institutional Review Board and TATRC after review by both the Principal Investigator and Medical Monitor. There was one adverse event during the last quarter, the event was deemed serious, not

related and unexpected due to testing that revealed terminal metastasis to the bone and adrenal gland. To date, there have been 5 adverse events, 4 were deemed serious and 1 event was not serious. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Three of the events were considered serious due to inpatient hospitalizations and one due to poor prognosis related disease progression. No deaths occurred and none of these adverse events were deemed to be study related.

Task #3: Continue "Defining the Genetic Basis of Heart Attack and Acute Coronary Syndromes in Military Service Women" at ICHP-WRI. (In collaboration with WRNMMC ICHP).

Methodology
This study will identify genes that affect susceptibility to heart attack in young military service personnel who have had a heart attack before the age of 55. Patients will be selected from the Department of Defense Serum Repository, which has millions of serum samples in storage. Cutting-edge technology will be used to isolate very small amounts of DNA that can be found in serum. More than 1,000,000 variations in the DNA will be tested. The ultimate objective is to identify new genes that increase risk for heart attack at an early age – such genes represent new targets for preventive or therapeutic interventions.

Status:
We have revised the study protocol, which will be initiated as a feasibility study. This modification in the study design will determine the feasibility of isolating and genotyping quality DNA from serum samples in the Department of Defense Serum Repository (DoDSR). For this proof-of-principal study we aim to: (1) assess the quantity and quality of DNA isolated from serum samples obtained from the DoDSR and (2) evaluate the performance of the obtained DNA on Affymetrix 6.0 SNP arrays containing 1.6 million markers. These preliminary studies will determine if we can use DoDSR DNA on high-density genetic marker arrays for future studies.

We continued our research and development work on whole-genome amplification of DNA samples and large-scale genomic research on these samples. The following abstracts were presented at the Association for Molecular Pathology November 2011 meeting. **Abstracts *were published* (See Appendix A)** in the Journal of Molecular Diagnostics.

- Voeghtly L, Croft DT Jr, Deyarmin B, Vernalis MN, Shriver CD, Ellsworth DL. Utility of whole genome amplification for assessing copy number variation with high density SNP arrays from formalin-fixed paraffin embedded tissue. Association for Molecular Pathology (AMP) 2011 Annual Meeting, 17-19 Nov 11, Grapevine, TX.

- Croft DT Jr, Voeghtly L, Patney HL, Shriver CD, Vernalis MN, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma for estimating copy number variation with high density single nucleotide polymorphism arrays. Association for Molecular Pathology (AMP) 2011 Annual Meeting, 17-19 Nov 11, Grapevine, TX.

Research and development work continued. Using laboratory samples that should be similar to the repository samples, call rates for all genomic DNA samples were all >97.90% (Table 6). Call rates for DNA isolated from serum were >93.00% and for DNA isolated from heparin plasma

were >95.7%. Samples from EDTA tubes that were whole-genome amplified did not perform well (~69-89% call rates). Serum samples from the DoDSR will be compared to these samples in the next period.

Table 6. Call rates on Affymetrix 6.0 arrays for DNA from various sources.

Sample	P/S	CQC	Call Rate
#1 Genomic	N/A	3.04	98.78
#2 Genomic	N/A	2.63	97.9484
#3 Genomic	N/A	2.43	97.9153
#5 Genomic	N/A	2.67	98.0477
#1 Serum Unamplified	Serum	0.72	93.051
#2 Serum Unamplified	Serum	2.32	97.7498
#3 Serum Unamplified	Serum	2.21	98.2793
#5 Serum Unamplified	Serum	2.25	97.85
#1 Serum WGA	Serum	2.19	96.1946
#2 Serum WGA	Serum	-0.05	84.71
#3 Serum WGA	Serum	2.15	96.1284
#5 Serum WGA	Serum	0.72	90.7346
#1 EDTA Unamplified	Plasma	0.47	89.74
#2 EDTA Unamplified	Plasma	3.31	99.01
#3 EDTA Unamplified	Plasma	-0.46	87.7895
#5 EDTA Unamplified	Plasma	0.43	91.4295
#1 EDTA WGA	Plasma	-0.07	77.9616
#2 EDTA WGA	Plasma	0.01	88.88
#3 EDTA WGA	Plasma	-0.03	68.63
#5 EDTA WGA	Plasma	-0.06	73.0311
#1 Heparin Unamplified	Plasma	1.67	95.7313
#2 Heparin Unamplified	Plasma	2.6	97.1873
#3 Heparin Unamplified	Plasma	2.6	98.84
#5 Heparin Unamplified	Plasma	2.41	98.1469
#1 Heparin WGA	Plasma	2.02	94.143
#2 Heparin WGA	Plasma	2.75	98.5109
#3 Heparin WGA	Plasma	2.21	97.3527
#5 Heparin WGA	Plasma	2.61	97.9815

Task #4: Initiate "Isolation, Amplification, and Genotyping of DNA from Serum Samples in the Department of Defense Serum Repository (DODSR): A Proof of Principle Study" protocol. (ICHP-WRNMMC in collaboration with WRI – see Task #3)

Methodology

The purpose of this proof-of-concept, laboratory-based study, utilizing serum samples obtained from the DoDSR is to: 1) assess the quantity and quality of DNA isolated from serum samples obtained from the DoDSR, 2) conduct whole-genome amplification of the serum DNA and evaluate the resulting whole-genome amplified DNA (wgaDNA), and 3) determine the feasibility of using the DoDSR wgaDNA on high-density genetic marker arrays for future studies. Fifty (50) orphan serum samples from individual service members that have been stored for different lengths of time and

meeting specific inclusion/exclusion criteria will be obtained from the DoDSR and will be anonymized at the source. This study will utilize two innovative technologies – whole-genome DNA amplification from stored serum specimens and whole genome characterization using advanced microarray technology – to determine the feasibility of using serum samples stored under the conditions of the DMSS/DoD Serum Repository for genome wide association studies.

Status: Received approved by WRNMMC DRP on 4 Mar 12. Submitted to TATRC on 9 Mar 12; USAMRMC ORP HRPO approval received 31 Jul 12. No Human Use determination. Initial discussions have begun with the DoDSR for selection and release of samples.

Task #4A: Initiate "Young Service Members with Myocardial Infarction" protocol.
Status: Pending results of Task #4.

Task #5: Initiate ~~"Predictors and Modifiers of the Natural History of Pre-Diabetes~~ "Exploring the Predictive Patterns of the Natural History of Pre-diabetes: Proof of Principle Study" protocol. (ICHP-WRNMMC in collaboration with WRNMMC Diabetes Institute and WRI). *Title of protocol changed per WRNMMC Scientific Review Committee.*

Methodology
The primary purpose of this prospective, observational, proof of principle study is to determine the feasibility of using a novel, point-of-care (i.e. home), multiple analyte test platform (Theranos) to study the temporal changes in five biomarkers related to glucose dysregulation, inflammation, vascular dysfunction, and immunity that can lead to diabetes and increased cardiovascular risk [insulin, leptin, high sensitivity Troponin T (hs-cTnT), high sensitivity C-reactive protein (hs-CRP), and ferritin]. A secondary purpose is to examine patterns of gene expression in peripheral blood in patients diagnosed with pre-diabetes who are entering into an intensive lifestyle modification program.

Up to 50 adult military healthcare beneficiaries (≥ 18 years) who met the screening criteria for pre-diabetes and have self-referred or been referred to the ICHP-CPP for CV risk reduction will be enrolled. Each participant will be provided a portable, home-based Theranos system and be asked to provide a fingerstick (FS) blood sample to the system at three specific times per week for 2 months pre-initiation and for the duration of their participation in the lifestyle change program. Blood samples will be collected prior to (2 months) and at the conclusion of the lifestyle program (8 months) to evaluate changes in gene expression and to determine changes in the biomarkers noted above. Blood samples will be collected again at 12, 24, and 36 months to determine if there are additional changes in the genetic markers and if the biomarkers are a measure of their dysglycemia.

A variety of statistical techniques will be used, depending on the level of measurement of the variables we are modeling (e.g., binomial, multinomial, continuous) to characterize the dynamic relationship between the analytes obtained by the Theranos system and 1) metabolic and CV risk and 2) advancement to diabetes and/or CVD.

Status: Protocol submitted to WRNMMC DRP 9 May 12. WRNMMC Scientific approval received 6 Sep 12. Pending IRB review on 11 Oct 12.

Task #6: Continue "Metabolic and Molecular Biology Studies in Surgical Interventions for Morbid Obesity" protocol at ICHP-WRI.

Methodology

This study represents a collaboration involving WRI, Windber Surgery Center, and WRNNMC. The purpose of the study is to characterize (1) molecular profiles in adipose tissue at baseline that are predictive of significant differences among individuals in rates of future weight loss, and (2) longitudinal molecular changes in peripheral blood that correlate with rates of weight loss in obese patients. Patients who are about to undergo laparoscopically placed adjustable gastric banding (LAGB) at the Windber Surgery Center as part of their physician-directed clinical treatment will be recruited for this study. Samples of subcutaneous and intra-abdominal adipose tissue will be collected by the surgeon at the time of surgery; blood samples will be collected prior to surgery and approximately every six months for 3 years. Ribonucleic acids (DNA and RNA) from tissue and blood samples will be analyzed for changes using highly sensitive technologies that permit genome-wide profiling. This research may provide information about molecular changes associated with weight loss and may improve our understanding of molecular events associated with obesity and CVD.

Approximately 500 participants will be recruited for this study from a convenience sample of patients presenting to the Windber Surgery Center at WMC as part of their physician-directed clinical treatment, and thus will be largely derived from local communities.

This study has been ongoing since 2006, funded through other sources at WRI. Adipose tissue and blood samples have been collected from 126 patients at 253 time points during the period January 2006 to December 2009. No molecular assays have been conducted.

Status: Protocol approved at WMC; USAMRMC approval received 15 June 12.

The protocol received second level approval from TATRC on June 20, 2012. Since that time, we consented patients to participate in this study and obtained blood and tissue samples. Fourteen lap-band patients were consented to participate in the research study; 32 follow-up blood samples were collected and 708 aliquots were processed and stored as summarized below:

Consented patients	14
Paxgene tubes for RNA	46
Adipose tissue (omentum & subcutaneous)	28
Plasma	200
Low-volume plasma	131
RBCs	157
NMR lipids	44
Leptin	44
Insulin	44
hsCRP	44

Key Research Accomplishments

- Better Adherence to Therapeutic Lifestyle Change Efforts (BATTLE) Trial
  - Main study data analysis complete; formative evaluation data analysis in progress
  - 1 manuscript submitted – in revision
  - 2 manuscripts in progress
  - 1 abstract accepted as podium presentation

- Non-Invasive Coronary Artery Disease Reversal (CADRe) Follow-Up Study
  - Data reconciliation completed
  - Statistical services contract in place
  - Final data analysis in progress
  - Publication plan in progress

- Cardiovascular Prevention Program (CPP):
  - 6 peer-reviewed publications from the CPP have been generated.
  - Translation of successful research findings into clinical care initiatives: using new approaches and tools to optimize care.
  - ICHP Database and Platform Creation for Data management: actively building infrastructure with robust plan for functionality and multiple protocol data capture capability.
  - Statistical support established and Publication plan in progress.
  - Combination clinical tracks used to promote health and aggressively combat metabolic dysfunction

- Validation of the ICHP Cardiovascular Risk Score
  - ICHP CV Risk score: continue data collection in CPP
  - Manuscript (in final stages)

- ZENITH Trial
  - Scientific approval received
  - Statistical services contract in place

- ICHP CPP Prospective Registry protocol
  - Scientific approval received

- Assessing Risk Factors for Cardiovascular Disease in Individuals with Traumatic Amputations
  - Modification to SOW approved and protocol approved at MRMC HRPO
  - Collaborative efforts begun

- Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal
  - Subject enrollment was 374 – 166 participants in the lifestyle change program, 140 subjects serving as the control group, and 68 participants enrolled in the Sub-study
  - One abstract presented at Obesity 2012 Annual Scientific Meeting in San Antonio, TX.
  - Two abstracts presented at the Association for Molecular Pathology (AMP) 2011 Annual Meeting in Dallas, TX

- Two abstracts accepted to upcoming meetings: American Heart Association Scientific Sessions 2012 in Los Angeles, CA and the American Society of Human Genetics Meetings in San Francisco, CA.
- Participation in the Program reduces levels of important biochemical risk factors for CAD, such as CRP and MIF (manuscripts in progress).
- Changes in gene expression mirror changes in many CVD risk factors – dramatic decrease during the first 12 weeks, then regression toward baseline from week 13 to 52.
- Most cholesterol and lipid homeostasis genes show a continual decrease in expression throughout the program similar to body weight.
- Medication use clearly does not affect gene expression, thus expression changes may be attributed to the lifestyle change program
- Genetic variation influences risk factor response
- Several SNPs show evidence of an influence on triglyceride response

- The Cardiovascular Risk Clinic (CRC)
  - Subject enrollment is 132; 114 participants remain active

- The Stress Therapy Empowering Prevention (STEP) program
  - Two abstracts presented at the National Consortium of Breast Centers Interdisciplinary Breast Center Conference in Las Vegas

- Isolation, Amplification, and Genotyping of DNA from Serum Samples in the Department of Defense Serum Repository (DODSR): A Proof of Principle Study
  - MRMC approval received
  - Collaboration dialogue begun

- Exploring the Predictive Patterns of the Natural History of Pre-diabetes: Proof of Principle Study
  - Scientific approval received

- Metabolic and Molecular Biology Studies in Surgical Interventions for Morbid Obesity
  - MRMC HRPO approval received

A Chronological Track Record of Research Productivity 2007 to 2012

1. Stress Apr 2007 PCNA— Nurses require tailored strategies to address their unique sources of and responses to stress. A needs assessment shows that nurses feel work stress, come to work even when sick or stressed, sleep too little, but report strong morale despite these conditions.

2. CPP Oct 2007 Army ACP—The CPP's integrative lifestyle change program produces substantial improvements in cardiovascular risk profile including BMI, blood pressure and lipids.

3. CPP Mar 2008 Circulation—An integrative lifestyle change program produces reliable changes in gene expression profiles.

4. CPP Apr 2008 PCNA—An integrative lifestyle change program can reduce nurse burnout.

5. Diet Jul 2008 SNE—Dietary guidelines do influence eating habits though the effect is slow to catch on. Innovative strategies such as integrative models of care may encourage more rapid adoption of healthy diet recommendations.

6. CPP Aug 2008 FHPC—An integrative prevention model is effective in managing patients with diabetes and pre-diabetes.

7. Stress Aug 2008 FHPC—Using the non-stigmatized portal of a lifestyle change program, highly stress nurses showed improvements in job satisfaction (37%), compassion fatigue (43%), burnout (59%), resulting from reduction in perceived stress (39%), enhanced sleep quality (30%), diminished sleepiness (34%), decreased fatigue (47%), and increase physical activity (69%).

8. Diet Oct 2008 FNCE—In conjunction with a therapeutic lifestyle change program, a Mediterranean diet may result in improved glucose control in diabetes (9 of 10 subjects) and reversal of pre-diabetes (10 of 14 subjects).

9. CPP Apr 2009 PCNA—In a therapeutic lifestyle change program, a nurse practitioner's assessment can identify new diagnoses in a substantial proportion of participants, particularly overweight/obesity (64%), insufficient sleep (63%), sleep apnea (48%), pre-diabetes (31%) and pre-hypertension (19%).

10. CPP Apr 2009 PCNA—Therapeutic lifestyle change using an interdisciplinary team approach successfully results in substantial weight loss and improvement in parameters of CVD risk.

11. CPP May 2009 QCOR—ICHP CV risk score outperformed Framingham risk score in correctly categorizing patients by coronary artery calcium score and CIMT.

12. Sleep May 2009 ATS—Using actigraphy to document no difference in total energy expenditure per 24 hours, nurses with long sleep lower BMI than those with short sleep.

13. Sleep Jun 2009 APSS—Exercise does not correlate with total sleep time (TST). A day with adequate exercise is not followed by a night with increased TST and a night with adequate sleep time is not followed by a day with increased exercise.

14. Stress Aug 2009 FHPC—High-stress subjects demonstrate greater daytime sleepiness, greater fatigue, higher BMI and higher lipoprotein-PLA2 than their low-stress counterparts. High levels of perceived stress adversely affect readiness.

15. Diet Oct 2009 FNCE—The Diet Habits Questionnaire is inadequate for assessment of compliance with a Mediterranean diet, with particular weakness in assessing intake of whole grains.

16. CPP Oct 2009 Obesity—Small amounts of exercise per week (the equivalent of 5 days/week of walking 20 min) can result in large reductions in weight gain and improved parameters of the cardiovascular profile, such as % body fat, glucose metabolism and cholesterol profile.

17. CPP Oct 2009 Obesity—Fast completers of the CPP were no different from slow completers with regard to weight loss, waist circumference, activity levels, insulin resistance and reversal of pre-diabetes.

18. Stress-Sleep Feb 2010 Stroke— Subjects with high levels of perceived stress had elevated BMI and increased waist circumference, as well as elevated glucose and Lp-PLA2 (strongly associated with stroke risk). High-stress subjects also demonstrated greater daytime sleepiness, greater fatigue, lower sleep quality, shorter sleep duration and a higher risk for sleep apnea than their low-stress counterparts.

19. Sleep May 2010 ATS Compared with short sleepers (total sleep time or TST $\leq$ 5 hrs/night), subjects with long sleep (TST $\geq$ 7 hrs/night) fell asleep more quickly, had better sleep quality, had less daytime sleepiness, and less fatigue. Long sleepers also weighed less, experienced lower stress, and had lower levels of the inflammatory marker hsCRP.

20. Stress May 2010 QCOR—At baseline, pre-diabetics with high levels of perceived stress have higher insulin levels, greater insulin resistance and higher percent body fat. Reduction of perceived stress among pre-diabetic subjects correlates with significant reduction in insulin levels, insulin resistance and indices of inflammation.

21. Sleep Jun 2010 APSS—Overweight participants in the CPP who improved their sleep quality showed greater weight loss compared to those whose sleep quality did not improve.

22. CPP Aug 2010 FHPC—Compared to those with normal CIMT, young soldiers with abnormal CIMT exercised less, snored more, were heavier, and had dyslipidemia and low vitamin D. There is a need to intervene in lifestyle behaviors at an early age.

23. CPP Oct 2010 ACNP—Subclinical hypothyroidism is not associated with increased risk for CVD, deranged lipid or glucose metabolism, or sleep symptoms.

24. Stress-Sleep Oct 2010 CHEST—Participants in the CPP who were able to decrease levels of perceived stress showed significant improvements in their sleep quality as well as improvements in cardiovascular risk markers including glucose metabolism and lipids

25. CPP Nov 2010 Army ACP—Comprehensive evaluation of young soldiers in the CPP indicates numerous maladaptive behaviors and risk factors that signal the need for early intervention to prevent CVD.

27. Stress Mar 2011 PCNA—It is feasible to incorporate abbreviated Mindfulness Training into a lifestyle change program with 1/3 of participants reporting successful use of the technique in their daily lives.

28. CPP Mar 2011 PCNA—The ICHP Risk Score dramatically improves CVD risk classification in women with diagnosed subclinical atherosclerosis.

28. Sleep Mar 2011 NSF—Subjects with hyper-insulinemia demonstrate an increased CVD risk profile and numerous indicators of disturbed sleep.

29. CPP Mar 2011 AFPH—Numerous health issues adversely affect the operational readiness of the Army Reserve Component. Many of the unhealthy behaviors are modifiable, deserve identification and are amenable to intervention.

30. CPP Mar 2011 AHA Epi—After one year in the CPP, participants showed 87 differences in metabolite profiles compared with controls indicating reduced CVD risk.

31. CPP Mar 2011 NCBC—STEP program description

32. CPP Mar 2011 NCBC—STEP (Step Therapy Empowers Prevention) program leads to improvements in lifestyle choices, quality of life, and indicators of overall health in patients with breast cancer.

33. CPP May 2011 QCOR—ICHP CV Risk Score (IRS) is more sensitive than Framingham Risk Score (FRS) in identifying subjects at increased risk for CVD. Of subjects with subclinical atherosclerosis by CIMT and low risk by FRS, the IRS appropriately up-scored risk level in 63% of subjects.

34. Sleep May 2011 ATS—The Berlin Questionnaire screens for subjects at risk for sleep apnea and CVD but also identifies traits of anxiety and perceived stress that may diminish the patients' ability to comply with CV risk reduction therapy.

35. Stress-Sleep Jun 2011 APSS—There are differences in levels of perceived stress, sleep quality, daytime sleepiness and fatigue between white and black subjects.

37. Sleep May 2012 ATS—Symptoms from sleep apnea differ by gender and race: women tend to feel fatigued while men tend to feel sleepy.

37. Diet Oct 2012 FNCE—Articulated dietary goals correlate with measured eating behaviors, indicating that subjects do recognize what eating behaviors require change.

38. Stress-Sleep Oct 2012 CHEST—Using a portable stress reduction technique in short intervals (Tension Tamer) may be an effective approach to improve cardiovascular risk through sleep improvement.

Reportable Outcomes

Published Manuscripts/Abstracts:

Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. SNPs associated with plasma triglyceride levels influence response during intensive cardiovascular risk reduction. (in preparation).

Ellsworth DL, Croft DT Jr, Weyandt J, Field LA, Patney HL, Burke A, Haberkorn MJ, McDyer FA, Jellema GL, van Laar R, Mamula KA, Vernalis MN. Intensive cardiovascular risk reduction induces sustainable changes in peripheral blood gene expression. (in preparation).

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Walizer EM, Vernalis MN. Does visual knowledge of increased risk for cardiovascular disease affect lifestyle change program adherence? (In preparation)

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Kashani M, Eliasson A, Bailey K, Vernalis M. Novel stress reduction technique improves sleep and fatigue. American College of Chest Physicians, 22 Oct 12, Atlanta, GA. (accepted)

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Miller EJ, Mamula KA, Leng L, Piecychna M, Vernalis MN, Bucala R, Ellsworth DL. Cardiovascular disease risk factor modification decreases HS-CRP and Macrophage Migration Inhibitory Factor (MIF): Influence of gender. American Heart Association Scientific Sessions 2012, 3-7 Nov 12, Los Angeles, CA. (accepted)

Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. SNPs associated with plasma triglyceride levels influence response during intensive cardiovascular risk reduction. American Society of Human Genetics, 6-10 Nov 12, San Francisco, CA. (accepted)

Eliasson A, Kashani M, Vernalis M. Sleepy on Venus, Fatigued on Mars? American Thoracic Society, 22 May 2012, San Francisco CA. (presented)

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Voeghtly L, Croft DT Jr, Deyarmin B, Vernalis MN, Shriver CD, Ellsworth DL. Utility of whole genome amplification for assessing copy number variation with high density SNP arrays from formalin-fixed paraffin embedded tissue. Association for Molecular Pathology (AMP) 2011 Annual Meeting, 17-19 Nov 11, Grapevine, TX. (presented)

Conclusions

Unhealthy lifestyle behaviors are linked to the development of CHD, as well as other chronic diseases. Projections based on combined CVD risk factor impact suggest that favorable lifestyle habits can substantially reduce CHD morbidity and mortality. We continue to demonstrate that comprehensive lifestyle interventions are remarkably efficacious in reducing CVD risk factors and, in many cases, are comparable to pharmacological interventions. Our research endeavors from this project continue to provide new information regarding strategies for improving the adoption of healthy lifestyle behaviors, the impact of lifestyle interventions on CVD risk, and the biologic mechanisms through which lifestyle changes exert their influence. Through this research, the DOD has a unique opportunity to identify and address adverse lifestyle behaviors and CVD risk factors early and make CV health a part of the military culture. A commitment to CV health could prevent cardiac events, reduce the need for costly procedures and hospitalization, improve quality of life and protect the investment of highly trained military personnel.

Appendix A
Manuscript/Abstracts

ORIGINAL RESEARCH

Stress Therapy Empowering Prevention (STEP): A Healthy Lifestyle Program for Breast Cancer Patients

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Purpose: Develop and implement a comprehensive program for lifestyle change, empowering breast cancer patients to manage stress effectively and improve their mental and physical health.
Method: Women with breast disease (or those at high risk) are offered a program of lifestyle change, consisting of a Healthy Lifestyle intervention for 3 months followed by monthly contact with a health coach. Instruction and demonstration provide information on exercise, nutrition, stress reduction, and mind/body health. Examinations are conducted at baseline, after completion of the intervention (3 months), at 1 year, and every 6 months for a period of 5 years.
Conclusion: Breast cancer has a significant emotional, psychological, and social impact and is often associated with high levels of stress that promote unhealthy behaviors causing weight gain, decreased physical fitness, and an increased risk for cardiovascular disease (CVD). Similar to CVD, research shows breast cancer susceptibility is also influenced in part by modifiable risk factors, suggesting that a healthy lifestyle program may lead to reductions in cancer risk and recurrence as well as improvements in mental health and quality of life. Through the Stress Therapy Empowering Prevention (STEP) program, breast cancer and high-risk patients are empowered with tools to focus on health promotion and optimization and maintenance of quality of life. Patients can improve physical and psychosocial factors in as little as 3 months, but long-term follow-up will determine if lifestyle changes result in improved clinical outcomes over time.

Extensive reports have documented the relationship between lifestyle changes and morbidity/mortality associated with cardiovascular disease (CVD). In particular, diet, physical activity, and stress are known to be associated with cardiovascular morbidity and mortality.[1-3] Similar to CVD, evidence has been mounting that breast cancer susceptibility is influenced in part by modifiable risk factors, such as body weight, diet, and physical activity, suggesting that a healthy lifestyle program may lead to a reduction in risk factors for CVD and breast cancer. Improving health and quality of life in patients with CVD and breast cancer will result in improved outcomes of care over the long term.

Early diagnosis and treatment are still vital to surviving breast cancer. Although an estimated 192,370 new cases of invasive breast cancer were expected in 2009, with approximately 40,170 deaths from the disease, incidence rates actually decreased by 2.0% per year,[4] likely due to advanced screening and early detection. In an effort to continue to lower incidence rates and improve long-term outcomes, studies of behavior modification in breast cancer patients are providing new information about how lifestyle factors affect survivorship as well as knowledge to help develop new, effective intervention programs to decrease breast cancer risk.[5-11]

The Stress Therapy Empowering Prevention (STEP) program is an innovative approach based on the concept that comprehensive lifestyle changes may have a meaningful impact on the risk for developing breast and cardiac disease. Given the advantages of a healthy lifestyle on both physical and emotional outcomes, cancer patients as well as those at high risk should be urged to address unhealthy behaviors. Our STEP model utilizes a specialized team comprising physicians, nurses, dietitians, licensed therapists, exercise physiologists, and stress management specialists who provide comprehensive strategies

that empower the participant to make healthier choices at an individual level. The program is an adjunct to treatment and care that participants receive from their personal healthcare providers. This combined effort allows for closer monitoring of each participant and coordination of care across the healthcare spectrum to achieve optimal health and quality of life.

METHODS

The overall goal is to recruit and evaluate approximately 500 women diagnosed with, or at high risk for, both breast and cardiac disease. The objectives of the study are to 1) test the efficacy of a healthy lifestyle intervention on reducing stress, sleep disturbances, and cardiovascular risk factors in both high-risk patients and patients diagnosed with breast disease; 2) evaluate the long-term benefit of an enhanced health coach intervention in promoting sustained wellness behaviors; and 3) examine molecular markers common to atherosclerosis and cancer to assess longitudinal changes and their relationship to disease development.

The STEP program has a 3-month healthy lifestyle intervention period during which participants meet once a week to learn the program guidelines, which include a low-fat, whole food nutrition plan based on the Mediterranean diet, aerobic and strength training exercises, stress management, and weekly mind/body health sessions. After the initial 3-month period, participants are contacted on a monthly basis by a health coach to ensure that program compliance is being maintained and to assist with long-term adherence. Participants are required to return to the center at the 1-year time point, and every 6 months thereafter for a period of 5 years, for testing and evaluation. Information collected includes perceived stress, sleep disturbance, psychosocial measurements, carotid ultrasound to measure carotid intima-media thickness, traditional risk factors (weight, blood pressure, body mass index [BMI], body composition), and biochemical assays.

To be eligible to participate, women must be 18 years of age or older with a diagnosis of breast disease (atypical hyperplasia, in situ carcinoma, or invasive breast cancer) or significant risk factors for developing breast disease such as previous biopsy, family history of breast disease, first pregnancy after the age of 30, early menstruation or late onset of menopause, or high risk of developing coronary artery disease (CAD) as indicated by having 1 or more of the following: family history of CAD, hypertension, diabetes, smoking, elevated blood lipids, sedentary lifestyle and obesity, or established clinically stable coronary disease.

Participants begin the program with an extensive physician visit to conduct a comprehensive risk assessment and develop a realistic lifestyle change plan. Participants are interviewed to assess sleep patterns, smoking status, cardiovascular and breast history, and medication use. The clinical exam includes height and weight measurements to calculate BMI (kg/m^2); blood profiles including thyroid-stimulating hormone, comprehensive metabolic panel, and fasting glucose and lipid panel; systolic and diastolic blood pressures; and psychological screening to evaluate mental health. Assessments are repeated at the end of the Healthy Intervention, at year 1, and every 6 months thereafter for a period of 5 years.

> "Our STEP model utilizes a specialized team comprising physicians, nurses, dietitians, licensed therapists, exercise physiologists, and stress management specialists..."

Following the initial examinations, participants attend an educational workshop designed to provide further instruction regarding the recommended lifestyle changes, followed by once-a-week sessions over a 3-month period. These sessions are tailored to ensure that each individual receives the appropriate education and experience needed to achieve success. Participants are required to complete a personal awareness log each week, which includes documentation of diet, exercise, and stress management frequency and duration, and a self-report of their mind/body session experience.

Blood samples are obtained from each consenting individual at baseline, at completion of the healthy lifestyle intervention, at 1 year, and every 6 months thereafter for a period of 5 years. From the blood samples, the following biochemical assays are analyzed: 1) lipoprotein subclass distributions determined by nuclear magnetic reso-

nance (NMR) spectroscopy; 2) stress/CVD biomarker panel: serum cortisol, insulin, leptin, highly sensitive C-reactive protein, lipoprotein(a), adiponectin, resistin, serum amyloid A, and vitamin D; and 3) breast disease–related panel: HER2/*neu*, tumor necrosis factor (TNF) alpha, and estradiol. In addition, blood is collected for isolating messenger RNA to determine changes in gene expression over the course of the study and identify new molecular markers associated with improved CVD biomarker risk profiles.

"Upon completion of the healthy lifestyle intervention (3 months), participants (n = 14) showed change in the desired direction for many risk factors."

RESULTS

Recruitment is being conducted primarily through newspaper and radio ads; distribution of patient information brochures; and speaking engagements at various community education events, physician offices, and support groups. Of 43 women who initially expressed interest in the program, 18 have enrolled thus far. Average age of participants was 65 years. Of the 18 participants enrolled in the program, 11 women had diagnosed breast disease (61%). In addition, of these same 18 women, 17 (94%) were also considered at high risk for developing CVD by having at least 1 documented CAD risk factor. Overall attendance was 88% during the initial 3-month on-site sessions. Four participants (22%) discontinued participation in the program, 3 due to personal, nonmedical reasons, and 1 due to breast cancer progression.

Upon completion of the healthy lifestyle intervention (3 months), participants (n = 14) showed change in the desired direction for many risk factors. Body weight (-1.8%, P <.05), BMI (-2.5%, P <.05), and perceived stress (-22.1%, P <.05) decreased significantly. Diastolic blood pressure (-8.4%, P <.08) and sleep quality (-26.5%, P <.06) showed near-significant changes. Most importantly, at the 1-year time point, perceived stress (n = 10, 8.2%, P <.05) and sleep quality (n = 9, -4.9%, P < .05) improvements were maintained, showing that these positive changes could be maintained over a longer period of time. In addition, though lacking statistical significance with our current sample size, triglycerides, systolic blood pressure, hostility, and depression all decreased at both time points (**Table**).

Based on self-reported exercise frequency and duration data, at 3 months participants on average were able to increase vigorous activity (heavy lifting, digging, aerobics, or fast bicycling) by 1.13 days/week, moderate activity requiring the participant to breathe somewhat harder than normal (carrying light loads or bicycling at a regular pace) by 1.56 days/week, and walking activity (including walking at work or home for recreation, sport, exercise, or leisure) by 1.63 days/week. At the 1-year time point, participants continued to show increased levels of activity for all measured categories; vigorous activity remained increased by 1.13 days/week, moderate activity by 1.13 days, and walking activity by 0.82 days when compared with baseline activity.

Lipoprotein subclass profiles will be assessed by NMR spectroscopy, which will quantify low-density lipoprotein particle number and size, and provide direct measurement of high-density lipoprotein and very low-density lipoprotein subclasses. Biochemical variables of interest regarding CVD risk, including insulin, leptin, lipoprotein(a), adiponectin, resistin, serum amyloid A, and TNF alpha will permit correlation of traditional CVD risk factors with nontraditional biomarkers to provide more information on the prevention and treatment of CVD. Vitamin D, HER2-*neu*, and estradiol will be analyzed to provide further insight into breast disease development and progression. Lower serum 25 (OH) D (vitamin D) concentrations may be associated with poorer overall survival and distant disease-free survival in postmenopausal breast cancer patients.[12] HER2-*neu* blood levels have potential as a tumor marker in breast cancer. Many studies have monitored circulating levels after surgery and reported that increasing HER2-*neu* levels can indicate recurrence of breast cancer earlier than clinical diagnosis.[13,14] Estrogens are believed to play a critical role in the etiology of breast cancer, and considerable evidence suggests that lifetime exposure to endogenous hormones, notably estrogens, promotes breast carcinogenesis.[15] Finally, cortisol levels, considered a major indicator of altered psychological states in response to stress, may provide information on short- and long-term stressors.[16]

Table Change in Selected Physical and Psychosocial Variables

Outcome	Baseline	3 Months	%Δ	*P**	1 Year	%Δ	*P**
Weight	180.5	177.3	-1.8	<.05	175.6	-2.7	.10
Body mass index	32.5	31.7	-2.5	<.05	31.5	-3.1	.38
Total cholesterol	201.1	200.2	-0.5	.90	203.4	+1.1	.73
Triglycerides	157.2	136.7	-13.0	.17	147.6	-6.1	.52
Systolic blood pressure	134.0	125.1	-6.6	.15	126.4	-5.7	.23
Diastolic blood pressure	79.6	72.9	-8.4	.08	76.3	-1.3	.28
Glycosylated Hgb	6.4	6.6	+3.1	.34	6.2	-3.1	.30
Fasting glucose	108.6	110.6	+1.8	.75	112.4	+3.5	.51
Depression	13.7	11.3	-17.5	.23	8.5	-38.0	.11
Hostility	6.4	4.8	-25.0	.16	5.7	-10.9	.33
Perceived stress	16.3	12.7	-22.1	<.05	11.7	-28.2	<.05
Pittsburgh sleep quality index	10.2	7.5	-26.5	.06	9.7	-4.9	<.05

**P* value based on repeated-measures analysis of variance.*

DISCUSSION

There are no proven substitutes for conventional cancer treatments such as surgery, chemotherapy, radiation, and immunotherapy; however, one approach to gaining a better understanding of how lifestyle change can enhance breast cancer survival is to develop studies that address several behavior and lifestyle factors within the same program. Research has shown that among women with breast cancer who had surgery and conventional treatment, those who learned to change their lifestyle through education focused on better nutrition, more exercise, and stress reduction were 68% less likely to die from disease over an 11-year period than those who did not.[17] Although the STEP study currently lacks long-term follow-up data, our program is examining the importance of helping breast cancer patients eat better, lose weight, improve strength and endurance, develop coping skills, and ultimately to improve their overall health and well-being. Participants in a STEP-style program feel better, both physically and emotionally. These observations suggest that the program has potential to improve their long-term overall risk profiles.

An important finding in our study was the struggle encountered in recruiting participants into the program. Obstacles to recruitment included out-of-pocket expenses, lack of local physician referrals, participant time constraints, and lack of knowledge among patients about the benefits of lifestyle change on quality of life or clinical outcomes. However, once women made the commitment to participate, surveys indicated a high degree of satisfaction with the program. Ultimately, issues encountered with recruitment affected our sample size, leading to difficulties in being able to effectively interpret preliminary data. In the future, we will continue to use best clinical judgment on when to approach appropriate patients based on past experience, to repeatedly offer to assist patients with addressing risk factors, and to educate healthcare providers about the STEP program to increase our sample size and provide additional data for analysis of the effects of lifestyle change on breast disease.

NUTRITION

Although the relationship between diet and breast cancer remains unclear, studies have shown that improved nutrition reduces the risk of several chronic diseases, such as obesity, diabetes, and heart disease, and that a healthy lifestyle improves overall quality of life.[18,19] Breast cancer patients who practice better nutrition are likely to derive benefit in terms of total mortality, similar to the general population. The Women's Healthy Eating and Living study showed that women who consumed a healthy diet and were physically active increased survival after diagnosis.[20] Patients who reported eating at least 5 servings of fruits and vegetables per day and performing 30 minutes of moderate walking 6 days a week reduced the probability of death by 50%.

The STEP program nutrition plan is based on

the Mediterranean diet and recommends eating vegetables; fruits; whole grains; lean protein sources such as fish, nuts, and olive oil; and minimizing the amount of red meat consumed. Participants are counseled to focus on eating more naturally occurring and fewer highly processed foods. Involvement of a registered dietitian helps to guide this process and provides the education, support, and long-term follow-up needed to meet the challenges of sustaining the recommended dietary changes.

The majority of studies of diet and breast cancer have examined the impact of body weight on survival. Most have observed that obesity at diagnosis is associated with poor prognosis.[21] Similarly, weight gain after diagnosis is common and is associated with mortality, disease recurrence, and development of comorbid conditions including diabetes and CVD.[22] Although some studies have shown that following a prudent diet alone, without adding physical activity, may not be associated with breast cancer survival,[5,23] a healthy diet has been shown to have beneficial effects on overall survival in conditions such as diabetes and heart disease, which are frequently seen in breast cancer patients.[24]

Participants in the STEP program were able to significantly decrease measures of obesity such as weight and BMI within the first 3 months of the program. Although these measures were not statistically significant at 1 year, they continue to remain lower than at baseline, suggesting that participants were successful in meeting or exceeding dietary compliance targets, thus preventing weight gain and promoting weight loss, which has been proven to be an effective strategy for improving overall quality of life and survival.

EXERCISE

Physical activity is as important as diet for achieving optimal weight and maintaining a healthy lifestyle. In studies examining the relationship between physical activity and the risk of breast cancer, a decrease in risk of approximately 25% was found among the most physically active women.[25] Similarly, in studies examining the effect of physical activity on breast cancer survival, some studies suggest that postdiagnosis physical activity may have great benefit. One study showed that after diagnosis, physical activity equivalent to walking 3 to 5 hours per week reduced mortality by as much as 50%.[26] Although the risk of developing comorbid conditions, including CVD, type 2 diabetes, fatigue, lymphedema, psychological distress, and poor quality of life, often persists in breast cancer survivors, recent studies have shown that physical activity can lower breast cancer risk and provide additional health benefits, such as decreased risk of stroke and type 2 diabetes, and improved longevity and quality of life.[27]

Most STEP participants achieved improvement in physical activity during the initial 3-month period, and many maintained these initial gains or continued to improve by the end of the first year. While most research demonstrates beneficial effects between physical activity and overall health, it is important to recognize that there is a risk-benefit ratio to exercise that may be different for each breast cancer patient. Utilizing a personalized plan might be most effective because it can be customized for different time periods, from prediagnosis through cancer treatment, based on individual needs and abilities. The STEP program develops each participant's activity plan based on an individual assessment completed by an exercise physiologist, but generally participants are encouraged to exercise aerobically for a minimum of 30 minutes per day, for a total of 3 hours of aerobic exercise each week. More intense exercise is permitted if medically appropriate and desired by the participant. Resistive or strength training exercise also is important, and if medically appropriate, participants were instructed to engage in strength training exercises 2 to 3 times per week. During the healthy lifestyle intervention portion of the study, hour-long supervised exercise sessions were scheduled.

The objectives of our exercise modality are to fully understand the importance and benefits of regular physical activity, to create a safe environment for exercise, and to encourage participants to properly monitor their own exercise program outside of the STEP program. These activities will assist with long-term adherence and allow the participant to achieve her own physical activity goals.

STRESS MANAGEMENT

Working with participants in the STEP program presents some unique challenges. These women have faced their mortality and live with the ongoing psychological stress of possible cancer recurrence.[28] A recent meta-analysis of 10 randomized controlled trials found that cancer patients who

participated in yoga interventions showed significant improvement in several psychological measures, including anxiety, distress, depression, and stress compared with wait-list controls.[29] For breast cancer survivors in particular, yoga has been shown to improve quality of life and emotional functioning.[30]

A mild form of physical activity, such as yoga or tai chi, may help to promote regular participation in physical activity. The therapeutic application of yoga enables participants to move slowly and safely, concentrating on relaxing their body while building flexibility, strength, and balance, which is especially important in breast cancer patients who may face additional barriers to more vigorous physical activity.[31] As emotional stress has been associated with decreased survival in breast cancer patients,[32] possibly by muting immune functions and accelerating the inflammatory response, stress management may offer a real survival advantage to cancer patients in addition to emotional benefits.

The STEP program's stress management specialist is a certified yoga therapist trained in techniques to provide participants with healthier ways to deal with the stress of living with a potentially life-threatening disease. The practice of yoga relies on physical postures to stretch muscles, focused breathing and meditation to minimize stress through visualization techniques, and guided imagery. Throughout the initial intervention, stress management sessions are held once a week. During these sessions, participants receive education and training in performing these techniques. The result is a relaxed body and a peaceful state of mind. Daily stress management practice was encouraged in the STEP program so that these techniques would be routine when patients are faced with a stressful situation.

MIND/BODY HEALTH

Women with breast cancer often exhibit emotional distress similar to posttraumatic stress disorder (PTSD).[33,34] In a recent study, among women who were recruited an average of 47 months following diagnosis of breast cancer, 38% had moderate to high anxiety, 22% had moderate to high depression, and PTSD was observed in 12%.[35] These findings show that the emotional impact of breast cancer can last for years following diagnosis. In addition, women lacking a social network had a significantly higher risk of breast cancer mortality than women with strong social ties to relatives, friends, and neighbors. Breast cancer patients often experience social isolation due to treatment, body image issues, or fatigue, which can have significant detrimental effects on psychological well-being by increasing levels of anxiety and depression. Therefore, it is important to recognize the signs of psychological distress in breast cancer patients and develop programs that effectively manage stress and mental health.[36]

The mind/body sessions in the STEP program are facilitated by a licensed therapist. These sessions are designed to create an atmosphere in which participants feel comfortable expressing their feelings and personal experiences. Since all STEP participants share common ground, individuals who self-disclose their experiences in dealing with breast disease encourage other participants to share their experiences as well. The overall purpose of the mind/body session is to create an environment where participants can experience belonging and the feeling of being connected. It is important to understand that these sessions are not group therapy—they are intended to facilitate making and sustaining healthy behaviors every day. Most of us know what we need to do to lead healthier lifestyles, but change is difficult to attain and sustain without ongoing support. This component upholds accountability, and the participants come to depend on each other for ongoing support.

CONCLUSION

In summary, lifestyle change interventions have proven to be beneficial to the vast majority of participants, but there are a limited number of studies that have examined the effect of combining several lifestyle behaviors into one comprehensive program to benefit breast cancer patients. The STEP program is a pioneer program that has combined the efforts of conventional treatment regimens with simple lifestyle changes, empowering breast cancer patients to actively manage their disease. As well-powered randomized controlled trials continue to define the effectiveness of lifestyle modification, hopefully more comprehensive programs will become available and eventually translate into improved care for breast cancer patients.

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Fatigued On Venus, Sleepy On Mars?

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Rationale

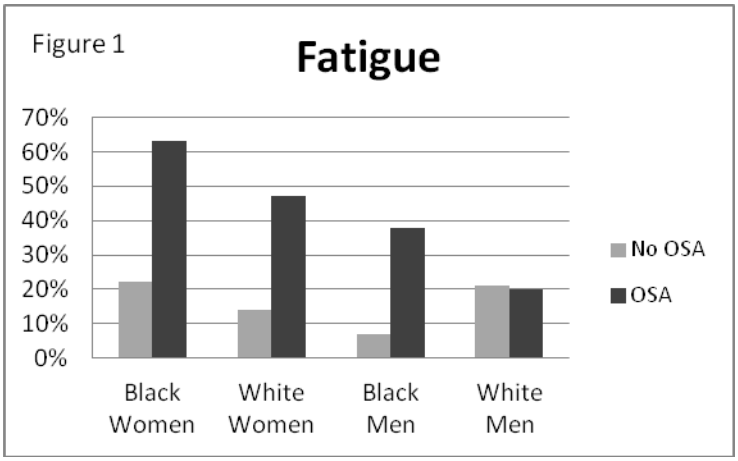
Subjective sleepiness and fatigue are recognized as separate symptoms which may occur singly, together, or may both be absent in subjects with obstructive sleep apnea (OSA). The inter-individual experiences of sleepiness and/or fatigue have recently been shown to be stable and trait-like with potential genetic causes. We sought to examine the vulnerabilities for sleepiness and fatigue in subjects with and without sleep apnea with special attention to the role of gender and race.

Methods

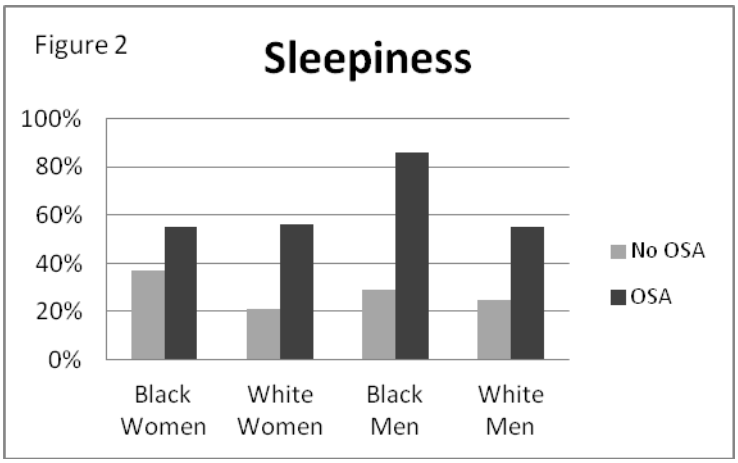
Consecutive subjects entering our heart health program completed a series of validated questionnaires. Thyroid function was tested in every subject. Sleepiness was defined by Epworth Sleepiness Scale $\geq$ 10 of 24 points. Fatigue was defined by the Stanford Fatigue Visual Analog Scale $\geq$ 5 of 10 points. The Berlin Questionnaire identified subjects as high or low likelihood for OSA. The two groups were compared using Fisher's exact test and two sample t-test as appropriate. For data analysis by race, comparisons were limited to White and Black categories as there were too few subjects for other comparisons.

Results

Of 295 consecutive subjects, 172 women (58%), there were 172 Whites, 105 Blacks, 13 Hispanics, 2 Asians and 3 others, with average age 57.4$\pm$12.7 years. Sleepiness was found in 129 subjects (44%) and fatigue in 90 subjects (31%). Berlin Questionnaires identified 159 subjects (54%) as high likelihood for OSA. There was no difference in thyroid function between subjects with and without a positive Berlin score (p=0.52). Without OSA present, numbers of subjects with fatigue were similar in women (15%) and men (20%), p=0.63. With OSA, fatigue was much more common in women (57%) compared to men (26%), p<0.001.



For sleepiness, there was no significant difference between the genders, p=0.43, but Black men did demonstrate a significant increase in subjects with sleepiness when comparing those with no OSA (29%) to those with OSA (86%), p=0.05.



Conclusions

Symptoms of fatigue and sleepiness are reported with different prevalence according to gender and race. Overall, women report fatigue more commonly in association with OSA than men. Black men experience sleepiness more commonly with OSA than other groups. These differences are not related to thryoid function. These findings deserve explanation with research that incorporates an objective measure of sleepiness and includes a broader range of variables such as the effects of total sleep time, co-morbid conditions, and medications.

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Perceived stress correlates with disturbed sleep: A link connecting stress and cardiovascular disease

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Abstract

The association between stress and cardiovascular disease (CVD) risk is becoming established. A mechanistic link clarifying the intermediate steps between the experience of stress and the development of CVD would support this association. We sought to examine the role of perceived stress as a factor associated with disturbed sleep with the goal of providing an explanation for the stress–CVD connection. We performed a cross-sectional analysis of data recorded by subjects at entry to our CVD prevention program. Data collection included questionnaire surveys, anthropometrics, and a CVD-relevant laboratory panel. Of 350 consecutively enrolled subjects (mean age 54.4 ± 12.4 [SD] years, 138 men, 39%), 165 (47%) scored above the mean for stress measures. These high-stress subjects displayed an increased cardiovascular risk profile including elevated body mass index (mean ± SD 31.1 ± 5.9 vs. 29.0 ± 5.9, $r_s = 0.175$), increased waist circumference (102 ± 17 cm vs. 98 ± 14, $r_s = 0.135$), and elevated high-sensitivity serum C-reactive protein (0.384 mg/dl vs. 0.356, $r_s = 0.109$). High-stress subjects also demonstrated greater daytime sleepiness (Epworth Sleepiness Scale: 10.4 ± 5.0 vs. 7.8 ± 4.8, $r_s < 0.316$), greater fatigue (fatigue scale: 5.4 ± 2.2 vs. 3.4 ± 2.4, $r_s = 0.484$), poorer sleep quality (Pittsburgh Sleep Quality Index: 8.5 ± 4.4 vs. 5.9 ± 4.0, $r_s = 0.416$), and shorter sleep duration (20 min less/24 h, r_s = negative 0.177) with a higher risk for sleep apnea (60% at high risk vs. 40%, $p = 0.003$) than low-stress subjects. High stress was associated with significant disturbances in sleep duration and sleep quality. Stress levels also correlated with daytime consequences of disturbed sleep. The stress–sleep connection may be an important mechanistic mediator of the association between stress and CVD.

Keywords: *Cardiovascular disease, perceived stress, risk factors, sleep, sleep quality, stress*

Introduction

An emerging body of evidence substantiates the observation that there is an association between stress and the occurrence of cardiovascular disease (CVD; Belkic et al. 2004; Rosengren et al. 2004). While the stress–CVD connection has been promoted and taught for decades, a number of difficulties have slowed a productive line of investigation in this area.

The impediments include how to define and measure stress (Cohen et al. 1983; Kocalevent et al. 2007), how to assess stress levels in a reproducible fashion over time (Kocalevent et al. 2009), uncertainty regarding causation between stress and CVD (Tindle et al. 2010), and the expense of performing a study to follow large numbers of subjects over a substantial period of time (Kadojić et al. 1999; Hamer et al. 2008). Furthermore, some studies appear to contradict the association between stress and CVD (Greenlund et al. 1995; Riese et al. 2000; Heslop et al. 2002a,b; Belkic et al. 2004). Nevertheless, the preponderance of studies to date supports the conclusion that stress, variously defined in a variety of approaches, does correlate with increased cardiovascular risk, both for heart disease (Melamed et al. 1992; Belkic et al. 2004; Rosengren et al. 2004; Brborović et al. 2009; Holden et al. 2010; Puustinen et al. 2010) and stroke (Everson et al. 2001; Surtees et al. 2008; Tsutsumi et al. 2009). Moreover, studies of depressive behaviors in female primates subjected to social stressors over a 4-year period have demonstrated significant acceleration of coronary artery atherosclerosis, suggesting a causal relationship between stress and CVD (Shively et al. 2008).

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To substantiate and explain the clinical observations correlating stress and CVD, it would be useful to clarify the underlying pathophysiology to outline mechanisms that link stress and CVD. It is clear that the hypothalamus–pituitary–adrenal axis plays a major role, by stimulating cortisol secretion, as do increased aldosterone and catecholamine levels, with a resulting detrimental effect on the cardiovascular system (Kubzansky and Adler 2010). It remains less clear what maladaptive conditions initiate the cascade of mediators that trigger these responses.

One mechanism was proposed in the Massa Lombarda Project, an epidemiological study including 7000 northern Italian adults (Bove et al. 2010). In a subset of 106 men and women, selected for older age, psycho-emotional stress and depression disorder were associated with the development of metabolic syndrome, a cluster of multiple cardiovascular risk states. Another study examined whether self-reported job strain was associated with early, potentially modifiable cardiovascular (CVD)-related health behaviors (Hellerstedt and Jeffery 1997). This study of 3843 randomly selected men and women employees of 32 worksites in Minnesota showed that work stress, defined as high demand and low latitude, was positively associated with smoking, smoking intensity, and high fat intake in men, and with body mass index (BMI) and smoking intensity in women. In 2008, the most sophisticated studies to date were published to describe the mechanistic links between stress and CVD (Chandola et al. 2008; Hamer et al. 2008). These studies used statistical models to assess the relative contributions of potential mediators of stress and CVD events. The Whitehall II study followed over 10,000 male and female civil servants for an average of 12 years (Chandola et al. 2008). The study showed that two factors, health behaviors and metabolic syndrome, accounted for around 32% of the effect of work stress on CVD. Another study that used statistical modeling was prospective and included 6576 healthy men and women followed over an average of 7.2 years (Hamer et al. 2008). Psychological distress was measured with the validated General Health Questionnaire, and actual CVD events (hospitalization for nonfatal myocardial infarction, coronary artery bypass, angioplasty, stroke, heart failure, and CVD-related mortality) were used as the main outcome. The investigators reported that behavioral factors explained the largest proportion of variance (approximately 65%), whereas pathophysiological factors accounted for a modest amount (C-reactive protein approximately 5.5%; hypertension approximately 13%).

The mechanisms proposed by these studies, while supported by objective data, fail to fully account for the observed relationship between stress and CVD. An often overlooked contributor to ill health and bad medical outcomes is sleep, with important sleep parameters including sleep duration and sleep quality. Failure to include the role of sleep in the stress–CVD connection is especially surprising in view of the substantial personal experience that all humans have of the ill effects of sleep deprivation and disrupted quality of sleep. Understanding the role of sleep as a possible link between stress and CVD is especially appealing because sleep behaviors can be taught and improved. Furthermore, it has been shown that improving sleep quality through the implementation of behavior modification does lower perceived stress levels (Eliasson et al. 2010). Disrupted sleep is thus a modifiable risk factor for stress levels and may therefore be, in extension, a modifiable risk factor for CVD.

We therefore hypothesized that high levels of perceived stress would correlate with disturbed sleep parameters. Such mechanistic link is indicated by substantial prior research showing that short sleep and disrupted sleep are associated with high risk for CVD (Heslop et al. 2002a,b).

Methods

The investigation was conducted with the approval of our institutional review board. The study design is a retrospective analysis of data collected on consecutive patients participating in a CVD prevention program at the Walter Reed Army Medical Center Integrative Cardiac Health Project (ICHP). ICHP is a cardiovascular prevention research center for the US Department of Defense. All data were retrospectively gathered and no blood samples were taken specifically for this study. The institutional review board, therefore, did not request informed consent from the study subjects.

Patients were self-referred or referred to the program by a health-care provider to improve habits of diet, exercise, sleep, and stress management. ICHP is accessible to military health-care beneficiaries including active duty service members, retirees, and dependents. The program, therefore, enrolls a broad spectrum of subjects including a variety of races and ethnic backgrounds, both genders, and a range of ages from 18 to 90 years. The typical patient entering the program is found to have two to four risk factors for CVD.

Upon entry, subjects are asked to complete a series of questionnaires to gather information on demographics, current symptoms, past and current medical conditions including medications and lifestyle habits. Among the questionnaires are the validated surveys to assess stress levels, sleep behaviors, sleep quality, and daytime symptoms from inadequate sleep. Data from the questionnaires are reviewed during a medical interview with a nurse practitioner who also performs a physical examination to include anthropomorphic measures.

Laboratory studies

Subjects gave blood for cardiac-relevant biochemical studies. For all blood samples, subjects were instructed to present to the laboratory between 06:00 and 08:00 h having fasted from 20:00 h the previous evening. The biochemical measurements on blood samples included a standard lipid panel with total cholesterol concentration, low-density lipoprotein (LDL) cholesterol concentration, high-density lipoprotein (HDL) cholesterol concentration, triglyceride concentration, as well as lipoprotein (a) and lipoprotein PLA2 concentrations. Measures of glucose metabolism include fasting plasma glucose concentration, insulin concentration, and hemoglobin A1C %. High-sensitivity C-reactive protein concentration (hsCRP) was also measured.

The laboratory studies were performed in the institution's certified central laboratory. The lipid panel was measured on a Roche Cobas c501 with appropriate reagents. The technique has documented traceability to the National Reference System for Cholesterol by performing a direct comparison with the cholesterol reference method using fresh human specimens, which cover the National Cholesterol Education Program (NCEP) medical decision points. The system has demonstrated the ability to meet the NCEP's performance criteria for accuracy and precision.

Perceived Stress Scale (PSS)

The PSS is one of the most widely accepted measures of stress (Cohen et al. 1983). This validated 14-item questionnaire asks the subject how often certain experiences of stress occurred in the last month and is designed to measure the degree to which situations in one's life are appraised as stressful. With item responses from 0 to 4, the range of possible scores is 0–56 with higher scores correlating with higher stress. The PSS is designed for use in community samples with at least a junior high school education. The items are easy to understand and the response alternatives are simple to grasp. Moreover, the questions are quite general in nature and hence relatively free of content specific to any subpopulation group. Score in the low 20s reveal moderate stress levels, while scores approaching 30 are substantial and concerning.

Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval (Buysse et al. 1989). Nineteen individual items generate seven component scores whose sum yields one global score with a range of 0–21. The psychometric and clinical properties of the PSQI suggest its utility both in clinical practice and research activities. A global score of greater than 5 indicates a poor sleeper. Sleep perturbations can be categorized by scores: 0–5 is a good sleep score; 6–10 shows mild sleep difficulty; 11–15 moderate sleep difficulty; and 16–21 severe sleep difficulty.

Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS) is the most widely used tool to estimate the subjective symptom of daytime sleepiness (Johns 1992). Subjects were asked to use a scale of 0–3 to estimate their likelihood of dozing in eight different situations in recent weeks. The individual scores were summed and possible scores range from 0 to 24. Sleepy subjects score 10 or higher and sleepiness can be categorized by scores: 10–14 as mild sleepiness, 15–19 as moderate sleepiness, and 20–24 as severe sleepiness.

Fatigue Scale

The Fatigue Visual Numeric Scale is borrowed from the Stanford Patient Education Research Center (see http://patienteducation.stanford.edu/research/vnsfatigue.html, accessed 1 July 2010). This fatigue scale asks subjects to express their experience of fatigue from 0 to 10 for the previous 2-week period. Subjects who circle 5–6 express mild fatigue, 7–8 moderate fatigue, and 9–10 severe fatigue.

Berlin Questionnaire

Of questionnaires available to screen patients for sleep apnea, the Berlin Questionnaire is one of the most commonly utilized and best validated (Netzer et al. 1999). As measured by the questionnaire, patients with persistent and frequent symptoms are considered to be at high risk for sleep apnea. Questions about symptoms demonstrated internal consistency (Cronbach correlations, 0.86–0.92). With a positive Berlin Questionnaire, sleep apnea was predicted with a sensitivity of 0.86, a specificity of 0.77, a positive predictive value of 0.89, and a likelihood ratio of 3.79.

Statistical analysis

Data are presented as mean ± SD. Two sample t-tests were used to compare continuous variables between groups, and categorical data were compared between groups using Fisher's exact test. Body habitus, sleep variables, and hsCRP did not satisfy assumptions of normality (as tested by the Shapiro–Wilk statistic) therefore, Spearman's correlation coefficient; (r_s) was used to examine the association of these variables with the PSS. All tests were two-tailed and p values <0.05 were presumed to represent statistical

Table I. Characteristics of the subjects according to perceived stress levels.

	All subjects ($n = 350$)	Low stress ($n = 185$)	High stress ($n = 165$)	t statistic	Degrees of freedom	p value
Age, years (mean ± SD)	54.4 ± 12.4	57.4 ± 11.5	51.1 ± 12.6	4.9	348	<0.001*
Race (%)						
Caucasian	134 (38%)	73 (39%)	60 (36%)			0.673†
African American	105 (30%)	53 (29%)	52 (31%)			
Hispanic	14 (4%)	5 (3%)	9 (5%)			
Asian	2 (1%)	1 (1%)	1 (1%)			
Others	96 (27%)	53 (29%)	43 (26%)			
Gender, male (% male)	138 (39%)	78 (42%)	60 (36%)			0.28†
PSS score (56 points max)	22.4 ± 8.1	16.3 ± 4.7	29.3 ± 5.0	−25.0	348	<0.001*

Notes: Values are mean ± SD or actual number of subjects in a category (with proportion). Statistical comparisons are between low-stress and high-stress subjects using the two sample t-test (or Fisher exact test as noted) with p values less than 0.05 representing statistical significance. Low-stress subjects were defined by a Perceived Stress Score less than the mean of 23 points, while high-stress subjects were defined by a score equal to or greater than the mean of 23 points; * denotes two sample t-test between low-stress and high-stress subjects; † denotes Fisher's exact test between low-stress and high-stress subjects.

significance. Data were analyzed using SPSS for Windows (v. 17.0, SPSS, Inc., (IBM), Chicago, IL, USA).

Results

We studied data from 350 participants entering ICHP's CVD prevention program. The mean age (±SD) of our participants was 54.4 ± 12.4 years, consistent with a spectrum of lifestyles from actively working to semi-retired and fully retired adults. Heavily represented racial categories were Caucasian and African American, but a substantial number of subjects identified themselves as mixed race or declined to pick a category. There was a majority of women (61%) in our study sample (see Table I).

As the mean PSS score was 22.4 points, we elected to define subjects with PSS scores of 23 or more points as belonging to the "high-stress" group and subjects with PSS less than 23 as the "low-stress" group. This allowed for analysis of data for nearly equal sized cohorts of high- and low-stress groups. While there are no defined ranges of "normality" or published degrees of severity based upon the PSS scores, the cut point of 23 does conform to a threshold value above which stress becomes a concerning issue from a clinical point of view in our experience within our program.

As summarized in Table I, there were no significant differences with regard to race or gender for high-stress and low-stress groups, though high-stress subjects were somewhat younger ($p < 0.001$).

As summarized in Table II, the cohort of subjects with high stress had a higher BMI (obese indices vs. merely overweight, $p = 0.001$) and larger measured waist circumferences. The biochemical measurement of hsCRP showed a positive correlation with perceived stress. The high-stress group also showed shorter total sleep times (20 min less per 24 h), poorer sleep quality, higher likelihood of sleep apnea diagnosis, greater sleepiness, and greater fatigue. The correlation between perceived stress and sleep quality is illustrated in Figure 1.

Several measurements ($n = 350$), not presented in the tables, showed no correlation with levels of perceived stress by Spearman's rank correlation. The lipid panel including total serum concentrations of

Table II. Correlations between perceived stress levels vs. anthropometrics, behavior scores, symptom scores, and laboratory values.

	Low stress ($n = 185$)	High stress ($n = 165$)	Correlation coefficient*	p value two-tailed
BMI (kg/m^2)	29.0 ± 5.9	31.1 ± 5.9	0.175	0.0011
Waist circumference (cm)	98 ± 14	102 ± 17	0.135	0.012
Total sleep time (hours/24 h)	6.4 ± 1.2	6.1 ± 1.5	−0.177	0.0011
Pittsburgh Sleep Quality Index (21-point scale)	5.9 ± 4.0	8.5 ± 4.4	0.416	<0.001
Berlin Questionnaire (% high risk for sleep apnea)	63/152 (41)%	72/120 (60%)		0.003†
Epworth Sleepiness Scale (24-point scale)	7.8 ± 4.8	10.4 ± 5.0	0.316	<0.001
Fatigue scale (10-point scale)	3.4 ± 2.4	5.4 ± 2.2	0.484	<0.001
hsCRP (mg/dl)	0.356	0.384	0.109	0.045

Notes: Values are mean + SD or proportion. Statistical comparisons are between low-stress and high-stress subjects using the two sample t-test (or Fisher exact test as noted) with p values less than 0.05 representing statistical significance. Correlation coefficients are derived from the Spearman's rank correlation coefficient (also called Spearman's rho) using a two-tailed test with $p < 0.05$ as the predetermined threshold of statistical significance. BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; * denotes Spearman's rho (r_s); † denotes Fisher's exact test.

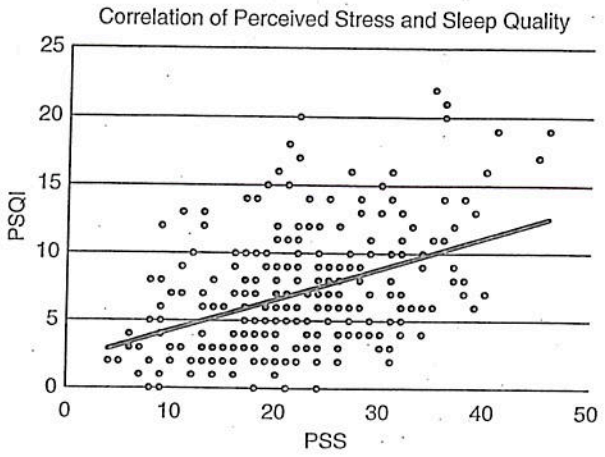

Figure 1. Using the Spearman correlation, there is a significant positive relationship between perceived stress scores (PSS) and scores on the PSQI, $r_s = 0.43$, $n = 274$, $p < 0.0005$.

cholesterol ($r_s = 0.04$), LDL cholesterol ($r_s = 0.004$), HDL cholesterol ($r_s = 0.015$), triglyceride ($r_s = -0.045$), and Lp (a) ($r_s = -0.013$) did not correlate with PSS. Likewise, parameters of glucose metabolism did not correlate with PSS, including fasting plasma glucose concentration ($r_s = 0.057$), HgA1C percentage ($r_s = 0.013$), and the homeostatic assessment model or HOMA ($r_s = 0.093$).

When sorted by gender, important differences were revealed. By *t*-tests ($n = 350$), women were slightly younger (54 ± 11 years vs. 58 ± 12 years, $F = 1.63$, $df = 348$, $p = 0.04$), had higher perceived stress scores (PSS $= 24 \pm 8$ vs. 20 ± 8, $F = 0.44$, $df = 348$, $p = 0.04$), had higher total serum cholesterol concentration (200 ± 37 vs. 176 ± 72 mg/dl, $F = 0.58$, $df = 343$, $p = 0.009$), and higher serum HDL cholesterol concentration (64 ± 22 vs. 48 ± 12 mg/dl, $F = 15.21$, $df = 343$, $p < 0.001$).

Discussion

The salient findings of this study are that increased levels of perceived stress were correlated with shortened total sleep time, worse scores for sleep quality, higher likelihood of sleep apnea, and worse daytime symptoms of sleepiness and fatigue. It is important to note that there were no concomitant correlations between perceived stress and lipid abnormalities or measures of glucose metabolism, two common risk factors for heart disease. It is known that normal values for lipids and glucose metabolism do not preclude an increased CVD risk. The finding that glucose and lipids did not correlate with stress in our study places greater weight on the role of sleep disruption in the development of CVD. In combination with numerous prior studies that connect short sleep and disturbed sleep with CVD (Heslop et al. 2002a,b), our correlations provide a mechanistic link to support the observed association between stress and CVD.

It is important to define stress and what is actually being measured with the PSS as it pertains to the current investigation. Because the PSS questions are general and free of content specificity, the instrument assesses subjectively experienced stress independent of an objective external stimulus or situation (Cohen et al. 1983). Personality aspects and resources of the subjects contribute to the total perceived stress score. The PSS correlates closely with trait neuroticism rather than the state of stress imposed. It therefore follows that trait neuroticism may be a pre-morbid characteristic of some good sleepers, who nonetheless manifest hyperarousal in response to stress and thus develop stress-induced insomnia (Basta et al. 2007; Fernandez-Mendoza et al. 2010).

The tools used to measure sleep in this study evaluate both sleep quality and sleep quantity. The high-stress group got an average of 20 min less sleep per night compared to the low-stress group. This may initially appear to be an inconsequential difference in sleep quantity. However, after only a few days or weeks, a substantial sleep debt can accrue, sufficient to affect mood, performance, and sense of well-being (Dinges et al. 1997; Drake et al. 2001). Furthermore, fatigue-inducing pro-inflammatory cytokines (interleukin-6 and tumor necrosis factor alpha) are negatively influenced by the quantity and quality of sleep (Vgontzas et al. 1999). CVD is a disease state stimulated and exacerbated by systemic inflammation. Prior research has also shown that insomnia with objective short sleep duration is associated with a higher risk for hypertension (Vgontzas et al. 2009a,b) and for type 2 diabetes mellitus (Vgontzas et al. 2009a,b), both major risk factors for CVD.

The Berlin Questionnaire focuses on an aspect of sleep quality. It is a validated instrument to quantify high vs. low risk for sleep apnea. The high-stress group with substantially higher BMI also has much higher odds of having sleep apnea. This finding is consistent with prior research that correlates increasing BMI with higher risk for sleep apnea (Newman et al. 2005). Explanations of these associations may include alternative theoretical models. Stress may stimulate maladaptive eating, leading to weight gain and subsequent development of sleep apnea. Alternatively, sleep apnea may disrupt the restorative functions of sleep (experienced as higher stress levels) and simultaneously disrupt hormonal regulation of hunger leading to greater calorie consumption and weight gain. These pathways toward greater risk of CVD warrant corrective attention at a time early in the cycle to preclude a downward spiral of health indicators.

Worse sleep quality as measured by the PSQI correlated with higher stress levels (Figure 1). Similarly, the ESS and fatigue scale, consequences

of the impact of poor sleep quality, correlated with higher stress levels. The finding that different tools showed worse scores with higher stress levels gives credibility to the observation linking poor sleep quality with high stress. Of course the challenge will be finding effective ways to improve sleep quality and consequent daytime symptoms, translating to improvements in CVD risk.

A novel aspect of our research is the use of the PSS and PSQI as tools to measure stress and sleep quality. There are few other studies that link perceived stress with poor sleep quality. There is one publication that utilized both the PSS and the PSQI in the same study (Strange et al. 2009). These coauthors investigated 220 pregnant women and found that PSS did not predict preterm birth and that preterm births were associated with lower daytime dysfunction scores on the PSQI. A PSS–PSQI connection was not reported in the study.

CVD is the leading cause of death in women, despite the cardiovascular protection afforded by their endogenous hormones and increased levels of HDL cholesterol (Wasserthiel-Smoller 2010). In our study, women were found to have significantly higher perceived stress scores than men. This finding may indicate that stress levels, specifically the measured PSS score, may be an important gender-relevant risk factor to survey, especially as a preventive strategy for improving women's health.

What cannot be determined in a cross-sectional study is causality. It cannot be inferred whether or not perceived stress causes deranged sleep or if poor sleep habits cause increases in perceived stress. It is possible that both perceived stress and sleep habits are worsened by another stimulus and that they respond in parallel to that stimulus. The relationship of perceived stress and disturbed sleep deserves further clarification, perhaps with a study providing an intervention aimed at stress or at sleep alone.

One limitation of the current study is that several indices were measured using subjective self-reports. Self-reported data included perceived stress levels, sleep quality, daytime sleepiness, and fatigue. However, the tools utilized to gather these indices were validated instruments with known performance characteristics and some of the data sought have no alternative ways of being measured. It may be useful in future studies to utilize objective measures such as a polysomnogram instead of the Berlin Questionnaire for sleep apnea and actigraphy as an objective measure of sleep quantity. Furthermore, strength of the current study is that actual measurements of height, weight, and waist circumference were used in place of self-reported values.

Our finding of correlation of perceived stress levels with sleep disruption adds to the growing body of evidence that stress may play an important role as a risk factor for CVD. Certainly the evidence to date is worthy of follow-up studies. A justifiable next study could examine the impact of stress management strategies and sleep improvement on incident CVD. Assessing maladaptive behaviors and physiological abnormalities associated with stress may allow for targeted intervention to promote vascular health.

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Performance of Whole-Genome Amplified DNA Isolated from Serum and Plasma for Estimating Copy Number Variation with High Density Single Nucleotide Polymorphism Arrays

Daniel T. Croft, Jr., Laura Voeghtly, Heather L. Patney, Craig D Shriver, Marina N. Vernalis, and Darrell L. Ellsworth

Introduction: Defining genetic variation associated with complex human diseases requires high-quality DNA from well-characterized patients. With the development of robust technologies for whole-genome amplification, sample repositories such as serum banks now represent a potentially valuable source of DNA for genomic studies and clinical diagnostics. We assessed the performance of whole-genome amplified (wga) DNA derived from stored serum/plasma for estimating chromosome copy number (CN) variation on high-density single nucleotide polymorphism (SNP) arrays.

Methods: Fresh serum and plasma samples were obtained from subjects who voluntarily agreed to participate in this study and gave written informed consent. DNA was extracted from 200 µl of serum or plasma using the QIAamp® DNA Blood Mini Kit. Genomic (g) DNA was isolated from peripheral blood mononuclear cells with the Puregene® DNA Purification Kit according to the manufacturer's protocol. Whole-genome amplification was then performed on 2.5 µl of serum/plasma DNA using the REPLI-g® whole-genome amplification kit. Genotypes were determined using Affymetrix GeneChip® Genotyping Analysis Software and CN variation was assessed with Genotyping Console™.

Results: Storage time and usage history did not affect DNA extraction or whole-genome amplification yields; however, samples that had been thawed and refrozen showed significantly lower call rates (73.9 ± 7.8%) compared to samples that had never been thawed (92.0 ± 3.3%) (P<0.001). Genotype call rates did not differ significantly (P=0.13) between wgaDNA from never-thawed serum/plasma (92.9 ± 2.6%) and gDNA (97.5 ± 0.3%) isolated from whole blood. Approximately 400,000+ genotypes were consistent between wgaDNA and gDNA; however, patterns of CN variation were highly discordant between serum/plasma wgaDNA and gDNA from the same patients. The CNV in the wgaDNA samples showed spurious regions of amplifications and deletions compared to the unamplified gDNA. These regions showed much larger areas of amplification and deletions across all the chromosomes compared to the unamplified gDNA CNV.

Conclusions: While use of stringent quality control requirements can facilitate the collection of quality SNP genotype data from wgaDNA, our data suggest that more advanced analyses, such as CN and loss of heterozygosity assessments, may be compromised due to spurious amplification during the whole-genome amplification process.

Citation:
Croft DT Jr, Voeghtly L, Patney HL, Shriver CD, Vernalis MN, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma for estimating copy number variation with high density single nucleotide polymorphism arrays. J Mol Diag 2011;13(6):781.

Racial Differences in Perceived Stress, Sleep Habits, and Daytime Symptoms

Arn Eliasson MD, Mariam Kashani CRNP, Jacqueline Hoffman MA, Marina Vernalis DO

Introduction: Racial disparities are important to understand in order to design effective programs for evaluation and intervention. We hypothesized that important racial differences exist in subjects enrolling in a heart health program.

Methods: The Integrative Cardiac Health Project (ICHP) is a heart health program that includes goals of improving sleep and stress management. At program entry, participants complete validated questionnaires, specifically the Berlin Questionnaire for sleep apnea, Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), fatigue visual analog scale (FVAS) and the Perceived Stress Scale. Subjects also submit to anthropomorphic measures and a cardiac-relevant lab panel. Differences between whites and blacks were compared using unpaired t-test and Wilcoxon rank sum test (2-tailed) as appropriate.

Results: Of 350 consecutive subjects (mean age 55.1 yrs, 28% men), there were 133 white (38%), 105 black (30%), 90 mixed race/undeclared, 14 Latino, and 8 others. For this analysis, only white and black subjects were considered. White subjects were somewhat older (57.4$\pm$12.6 yr vs 52.1$\pm$12.4, p=0.001) and included more men (47% v 34%, p=0.04). BMI was similar between groups (29.5$\pm$5.1 kg/m^2 vs 30.6$\pm$6.6, p=0.18). White subjects had lower perceived stress (PSS=19.4$\pm$9.6 vs 23.6$\pm$6.8, p<0.001), better sleep quality (PSQI=6.1$\pm$4.1 vs 7.1$\pm$3.9, p=0.05), and less daytime sleepiness (ESS=8.0$\pm$4.9 vs 9.8$\pm$5.0, p=0.01). White subjects tended to have less fatigue (FVAS=3.9$\pm$2.5 vs 4.5$\pm$2.4, p=0.08) and longer sleep duration (20 min longer per night, p=0.07). However, there was no difference in sleep latency (24.4 min vs 23.0, p=0.85) or likelihood for sleep apnea (Berlin positive 44% vs 51%, p=0.40).

Conclusions: There are important differences in levels of perceived stress, sleep quality and daytime sleepiness between white and black subjects in our program. These differences deserve explanation and may be valuable in designing interventions tailored for specific groups.

Citation:
Eliasson A, Kashani M, Hoffman J, Vernalis M. Racial differences in perceived stress, sleep habits, and daytime symptoms. Sleep 2011;34:A262.

Novel Tool Improves CV Risk Stratification and Guides Therapy

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Background: Accurate risk assessment is of critical importance to any cardiovascular (CV) disease prevention program. Risk stratification tools enable providers to implement appropriate therapies.

Objective: We sought to compare the performance of the Framingham Risk Score (FRS) with a CV Score previously validated by the Integrative Cardiac Health Project (ICHP) in a cohort of subjects with known subclinical atherosclerotic disease by abnormal carotid intima-media thickness (CIMT) measurement.

Methods: Consecutive subjects (n=93) identified with subclinical atherosclerosis by abnormal CIMT ($\geq 75^{th}$ percentile by age/gender) were enrolled in a 6-month CV risk reduction program. Subjects were assessed for past medical history, family history of CV events, anthropometrics and a cardiac-relevant lab panel. FRS and ICHP CV Risk Score were calculated for each individual and were compared. The ICHP CV Risk Score incorporates additional factors such as family history of CV events as well as novel risk factors. All scores were categorized as low, medium and high for CV risk.

Results: In 93 consecutive subjects, mean age was 53.1$\pm$11.13 yrs, 59% women, 47% African-American, 46% Caucasian, 3% Latina, 1% other. Diagnosis of diabetes was present in 13% of subjects. Means: BMI=31.2$\pm$5.3 kg/m^2 , WC=100.2$\pm$13.6 cm, fasting glc=99.1$\pm$35.9 mg/dL, insulin 15.6$\pm$14.4 ug/dL, Tchol=194.9$\pm$42.3 mg/dL, LDL 114.5$\pm$34.0 mg/dL, HDL 56.2$\pm$18.5 mg/dL, TG 117.3$\pm$66.3 mg/dL, Lp(a)=86.5$\pm$92.3 mg/dL, CRP 0.4$\pm$0.6 mg/dL.

By FRS, 12 (14%) subjects scored high risk, 11 (12%) scored medium and 70 (75%) scored low risk. By ICHP CV Risk Score, 4 (36%) of the FRS medium upscored to high risk and 47 (67%) of the FRS low upscored to medium risk. In total, 63% upscored to an appropriately higher risk category by using the ICHP CV Risk Score.

Conclusion: In a population with documented subclinical atherosclerosis and unremarkable conventional risk factor profiles, the ICHP CV Risk Score appeared to be more sensitive in identifying subjects at risk. The ICHP CV Risk Score may be a more discerning tool to guide risk reduction therapy in a prevention program.

Citation:
Kashani M, Eliasson A, Bailey K, Vernalis M. Novel tool improves CV risk stratification and guides therapy. Circ Cardiovasc Qual Outcomes. 2011;4:AP88.

Utility of Whole Genome Amplification for Assessing Copy Number Variation with High Density SNP Arrays from Formalin-Fixed Paraffin Embedded Tissue

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Introduction: The ability to obtain sufficient high quality DNA from archival formalin-fixed paraffin embedded (FFPE) tissue often limits genomic analysis for researchers and clinicians alike. Of numerous methods developed to optimize the quantity of DNA extracted from FFPE tissues, whole genome amplification (WGA) has become a robust and reliable technique for obtaining sufficient genomic material for a variety of molecular applications. Previous studies suggest that DNA obtained from FFPE samples may be used on high-density single nucleotide polymorphism (SNP) arrays to provide information on SNP genotypes, chromosome copy number (CN), and loss of heterozygosity, but spurious results occur with insufficient DNA template.

Methods: We examined the feasibility of assessing chromosome CN variation using whole-genome amplification on DNA extracted from FFPE tissue, as well as fresh frozen (FF) tissue in OCT, and high-density Affymetrix GeneChip® 500K SNP Mapping Arrays. Genomic DNA was extracted from microdissected regions (approx 2.9 mm^2) of human tissue preserved in paraffin using the GenomePlex® Tissue Whole Genome Amplification Kit (Sigma®) and from human FF tissue using QiaAmp® DNA Mini Kit (Qiagen®). Whole-genome amplification was then performed on 1.5 µl of FF or FFPE DNA using the REPLI-g® whole-genome amplification kit (Qiagen®). Genotypes were determined using the Dynamic Model Mapping Algorithm in the Affymetrix GeneChip® Genotyping Analysis Software (GTYPE 4.0) package and CN variation was assessed with Genotyping Console™ (Affymetrix).

Results: Acceptable genotyping call rates were obtained for all unamplified DNA samples (96.3 ± 1.5%) and wgaDNA samples (93.3 ± 1.6%) from FF tissue. Call rates were significantly lower; however, for wgaDNA samples from FFPE (67.5 ± 5.1%) ($p<0.001$). Assessment of CN variation was highly consistent between unamplified and whole-genome amplified FF samples, but was clearly discordant between amplified FF and amplified FFPE samples.

Conclusions: These results indicate that FF tissue, even if whole-genome amplified, is useful for genome-wide SNP genotyping and determining chromosome CN variation, but large discrepancies are likely to occur when using whole-genome amplification on DNA isolated from FFPE. CN variation may be affected by uneven amplification of the genome with small quantities of suboptimal DNA template extracted from FFPE samples.

Citation:
Voeghtly L, Croft DT Jr, Deyarmin B, Vernalis MN, Shriver CD, Ellsworth DL. Utility of whole genome amplification for assessing copy number variation with high density SNP arrays from formalin-fixed paraffin embedded tissue. J Mol Diag 2011;13(6):780.

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Cardiometabolic risk reduction in an intensive cardiovascular health program

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Cardiovascular disease;
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Lifestyle modification;
Low-fat diet;
Exercise

Abstract *Background and aims:* Insulin and leptin are important markers of insulin resistance and vascular inflammation in metabolic and cardiovascular diseases. This study evaluated changes in circulating levels of insulin and leptin during a cardiovascular health program to improve our understanding of cardiometabolic risk reduction.
Methods and results: Participants ($n = 76$) completed a prospective, nonrandomized program designed to stabilize or reverse progression of coronary artery disease through dietary changes, exercise, stress management, and group support. Controls ($n = 76$) were matched to participants based on age, gender, and disease status. Traditional cardiovascular risk factors were assessed at baseline, 12 weeks, and 52 weeks by standard methods. Dietary data were collected by 72-h recall and evaluated by Food Processor® v8.4.0. Ultrasensitive insulin and leptin levels were measured by radioimmunoassay. Participants successfully reduced their total caloric intake from >2000 calories per day to ~1700 calories per day ($p < 0.05$ compared to controls), lowered daily fat intake by >60% ($p < 0.001$ compared to controls), and increased carbohydrate intake by >30% ($p < 0.001$). Repeated-measures ANOVA indicated significant beneficial changes ($p < 0.001$ compared to controls) in plasma insulin (−19%) and leptin (−33%) during the lifestyle program, as well as improvement in traditional cardiovascular risk factors. Response was similar between men and women for most risk factors and was not markedly influenced by medication use.
Conclusion: Lifestyle changes focusing on diet, physical activity, and stress reduction can successfully modify both cardiovascular and metabolic risk factors, with the potential to mediate cardiometabolic risk through beneficial anti-inflammatory and anti-oxidative effects on the vasculature.

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Introduction

Insulin and leptin represent two important and well-characterized markers of insulin resistance and vascular inflammation in metabolic and cardiovascular diseases (CVD). Insulin is a polypeptide hormone that affects the vascular endothelium by modulating glucose homeostasis and glycogen synthesis [1]. Fasting insulin levels have increased dramatically in non-diabetic adults over the past two decades, often developing as a consequence of resistance to the action of insulin in peripheral tissues [2]. Hyperinsulinemia has been linked to dyslipidemia, impaired glucose regulation, and hypertension [3], as well as overall risk for cardiovascular mortality [4].

Leptin is an adipocytokine secreted by white adipose tissue that functions mainly in energy balance and metabolism, but plays an important role in vascular physiology through interactions with the vascular endothelium [5,6]. High circulating levels of leptin may accelerate atherosclerosis and contribute to CVD risk by inducing oxidative stress on endothelial cells [7] and impairing arterial reactivity [8]. Clinical studies have shown that high leptin contributes to CVD risk in the general population and is associated with myocardial infarction and coronary events, independent of traditional cardiovascular risk factors [9,10].

Insulin resistance, vascular inflammation, and oxidative stress play important roles in endothelial dysfunction. Pharmacologic therapies to improve endothelial function show marked variability in their ability to lower circulating markers of inflammation [11], and are often used in combination to be most effective in reducing inflammation and oxidative stress. An alternative approach for treating patients with high cardiovascular risk involves lifestyle modification to reduce traditional CVD risk factors and slow or reverse progression of coronary atherosclerosis [12]. Lifestyle programs focusing on nutrition and exercise can improve endothelial function and enhance insulin sensitivity, in part by reducing markers of systemic vascular inflammation and insulin resistance [13].

Insulin and leptin have important effects on vascular biology, but may function through different molecular pathways — insulin through metabolic pathways and leptin through inflammatory and thrombogenic factors [14]. We investigated the impact of an intensive cardiovascular health program on circulating levels of insulin and leptin to improve our understanding of cardiometabolic risk factor reduction by (1) measuring changes in physiological risk factors for CVD throughout a year-long cardiac health program and (2) assessing response of insulin and leptin and relating changes in these inflammatory markers to improvement in vascular health.

Methods

Study population

The intervention group consisted of 76 white men and women who completed a prospective, nonrandomized program to stabilize or reverse progression of coronary artery disease (CAD) through dietary changes, exercise, stress management, and group support. Eligibility criteria were (1) a diagnosis of CAD, including acute myocardial infarction, bypass surgery, stent placement, stable angina, angioplasty, or evidence of $\geq$50% luminal narrowing on coronary angiogram; or (2) two or more CAD risk factors such as high blood pressure (BP) defined as systolic pressure $>$140 mm Hg or diastolic pressure $>$90 mm Hg, high total cholesterol ($>$200 mg/dL), physician diagnosed diabetes, obesity — body mass index (BMI) $\geq$30 kg/m^2, or family history of heart disease in parents or siblings. Physician approval, motivation to commit to following the guidelines of the program, and successful abstinence from smoking for at least three months prior to enrollment also were part of the acceptance criteria.

Controls ($n = 76$ white men and women) were matched to participants based on gender, age at baseline within five years, and CAD status (overt CAD or risk factors) using a prospective individual matching strategy to achieve a balanced distribution of risk factors between intervention participants and controls in nonrandomized clinical trials [15]. Controls receiving only standard care from their primary physicians underwent identical examinations at baseline, 12 weeks, and 52 weeks, but did not participate in the program or receive healthy lifestyle information.

This study was approved by the Institutional Review Board at Windber Medical Center. All participants voluntarily enrolled in the program and provided written informed consent.

Intervention

The lifestyle program included four components: (1) low-fat vegetarian diet ($<$10% of calories from fat); (2) 180 min/week of moderate aerobic exercise; (3) 1 h of stress management each day; and (4) two 1-h group support sessions per week for the first 12 weeks and one group session per week during the remainder of the year [16]. Adherence was self-reported by summarizing diet (fat, carbohydrate, protein intake), exercise (frequency and duration), stress management (frequency and duration), and group support (frequency of meeting attendance) for each day. Program staff reviewed compliance forms weekly and provided immediate feedback to participants on progress and guidance for improving adherence.

From January 2004 to February 2009, approximately 35 participants or controls were enrolled each year in separate cohorts of $\sim$12 individuals per cohort. The dropout rate was $\sim$32% ($n = 53$) among participants in the program, likely attributable to the magnitude of lifestyle changes required.

Physiological measures

Data collection and reporting followed recommendations of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) group [17]. Clinical examinations conducted by physicians or trained personnel at baseline, 12 weeks, and 52 weeks collected information on age, gender, ethnicity, smoking status, cardiovascular history, and medication use. Height and weight measurements were used to calculate BMI. Blood pressure was recorded using a mercury sphygmomanometer on the arm of

seated, relaxed subjects. General endurance was determined by a graded treadmill exercise test that estimated the volume of oxygen each participant could consume (VO_2 max; ml/kg/min) based on exercise intensity, duration, and body weight (Bruce score) [18]. Assays for standard high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol, and triglycerides were conducted using the AEROSET™ clinical chemistry system (Abbott Laboratories, Abbott Park, IL).

Insulin and leptin measurements

Fasting blood samples for standard insulin and leptin analysis were obtained at each examination and placed directly on ice. Within 1 h of collection, plasma aliquots were isolated by centrifugation and stored at −80 °C. Ultrasensitive insulin (μU/ml) and leptin (ng/ml) levels were measured in duplicate on freshly thawed plasma samples by radioimmunoassay (Millipore, Billerica, MA) at the Johns Hopkins Bayview Clinical Research Unit. Inter-assay coefficients of variation (CV%) were 3.27 for insulin and 3.81 for leptin.

Dietary composition

Participants and controls completed a self-reported 72-h dietary recall questionnaire at each examination, recording their total dietary intake for breakfast, lunch, dinner, and snacks over three consecutive days. Participants reported specific food items and drinks consumed, portion sizes, preparation methods, and location (home or away). Food Processor® v8.4.0 (ESHA Research, Salem, OR) was used to determine daily caloric intake and nutrient composition.

Statistical analysis

Statistical analyses were conducted using SPSS version 15.0 and JMP® version 9.0; p values <0.05 were considered significant. Prior to analysis, normality of the outcome data was determined by Lilliefors test, and natural log-transformations were used for variables with non-normal distributions. Potential differences in baseline measures among participant cohorts and among control cohorts were examined by analysis of variance (ANOVA). As no significant cohort-to-cohort variability at baseline was detected, all intervention and all control cohorts were, respectively, combined in subsequent analyses.

An independent samples t-test, or nonparametric Mann–Whitney U test if data remained non-normally distributed after natural log transformation, was used to compare baseline characteristics between intervention participants and controls. Repeated-measures ANOVA was used to compare changes in CVD risk factors at 12 weeks and 52 weeks between intervention and control groups. Independent samples t-tests (two-tailed) then identified differences in risk factor response from baseline to week 52 between the intervention and control groups. For each variable, differences in response between men and women were assessed by two-factor repeated-measures ANOVA using a Bonferroni adjustment. As above, t-tests compared baseline to week 52 changes between groups, by gender. To examine the potential confounding effects of medications on insulin and leptin response, sub-group analyses were conducted that excluded participants who changed the brand or dosage of any medication known to affect insulin, leptin, and/or lipid levels through (1) main (intended) effects or secondary (side) effects, or (2) main effects only.

Results

Baseline measures

At baseline, participants showed higher plasma insulin, % carbohydrate intake, BMI, and triglycerides, but lower % fat consumption and exercise capacity than controls despite the prospective matching strategy (Table 1). Insulin values did

Table 1 Cardiometabolic risk factors, dietary components, and physiological measures at baseline for participants and controls in the cardiac lifestyle program.

Measures	n	Controls	Participants	P[a]
Cardiometabolic risk factors				
Insulin (μU/ml)	150	14.3 ± 7.1	18.1 ± 10.2	0.012[b]
Leptin (ng/ml)	152	19.0 ± 17.2	23.5 ± 18.6	0.059[b]
Dietary components				
Calories (kcal/day)	114	1736 ± 582	2095 ± 776	0.056
% Carbohydrate intake	114	49.5 ± 9.2	54.0 ± 12.2	0.010[b]
% Fat intake	114	32.5 ± 8.5	28.8 ± 10.2	0.037
% Protein intake	114	16.7 ± 3.9	16.8 ± 6.3	0.591[b]
Physiological measures				
Age (years)	152	60.6 ± 7.6	60.6 ± 7.6	0.992
BMI (kg/m^2)	152	28.5 ± 4.5	32.9 ± 7.2	<0.001
Systolic BP (mm Hg)	146	132.0 ± 16.3	136.2 ± 16.9	0.119[b]
Diastolic BP (mm Hg)	146	78.6 ± 10.1	81.0 ± 10.1	0.238[b]
HDL cholesterol (mg/dl)	152	49.4 ± 13.0	45.5 ± 13.4	0.057
LDL cholesterol (mg/dl)	142	108.1 ± 33.8	112.5 ± 39.1	0.590
Total cholesterol (mg/dl)	152	185.3 ± 42.7	194.8 ± 48.2	0.200
Triglycerides (mg/dl)	152	143.5 ± 97.8	175.9 ± 94.2	0.004
Exercise capacity (Bruce score)	122	9.3 ± 2.9	6.6 ± 2.1	<0.001

Data are presented as mean ± SD; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a] Tested by 1-factor ANOVA by cohort type.

[b] Tested by a nonparametric Mann–Whitney U test because data was not normally distributed after natural log transformation.

not differ significantly between participants who completed the program (graduates) and those who dropped out; however, leptin levels were higher (30.0 ± 18.4 versus 23.5 ± 18.6, $p < 0.05$) among dropouts. Dropouts also tended to be younger (55.1 ± 11.0 versus 60.6 ± 7.6, $p < 0.01$) and have lower systolic BP (130.4 ± 18.7 versus 136.1 ± 16.7, $p < 0.05$) than graduates. None of the risk factors differed between participants excluded from the analysis due to non-matching and those included in the study.

Changes in cardiometabolic risk factors

Participants in the cardiovascular health program experienced significant beneficial changes in plasma insulin and leptin (Table 2). Insulin levels declined ~19% in participants ($p < 0.001$ versus controls), while leptin levels decreased 33% ($p < 0.001$ versus controls). In contrast, both insulin (+4%) and leptin (+6%) increased in controls over one year.

Changes in dietary composition

Controls showed no significant change in any dietary component; whereas, participants reduced total caloric intake from >2000 calories/day to ~1700 calories/day (-18%) ($p < 0.05$ versus controls). Similarly, participants lowered daily fat intake by >60% ($p < 0.001$ versus controls) and, on average, maintained a total fat intake of ~11% of calories (Table 2). Carbohydrate intake increased by >30% among participants ($p < 0.001$ versus controls), while dietary protein remained unchanged.

Response of traditional CVD risk factors

Participants achieved a 9% reduction in BMI by the end of the year ($p < 0.001$ versus controls), a 6% reduction in diastolic BP ($p < 0.05$), and a 37% increase in physical fitness ($p < 0.001$), all significant improvements. Systolic BP and total cholesterol improved significantly from baseline to 52 weeks, but the degree of change did not differ between participants and controls. HDL decreased significantly by the end of the year, but overall change was not significantly different from controls.

Gender differences in response

Gender was not a significant factor for changes in any CVD risk factor from baseline to 52 weeks among controls. Men and women participating in the program showed similar significant improvement for insulin and leptin, nearly identical changes in diet (Fig. 1), and equivalent changes in BMI and physical fitness ($p < 0.001$) compared to controls after one year. Response for diastolic BP also was similar between genders — significantly different from baseline at 12 weeks and 52 weeks in participants, but the magnitude of change was not significantly different from controls. Triglyceride levels dropped significantly (−20%) among male participants ($p < 0.05$ versus controls), but in women, triglyceride response did not differ between participants and controls, and actually increased ~5% among female participants from baseline to the end of the year.

Table 2 Cardiometabolic risk factors, dietary components, and physiological measures for participants and controls in the cardiac lifestyle program at baseline, 12 weeks, and 52 weeks.

Measures	Controls ($n = 76$)				Participants ($n = 76$)				Between group P[a]
	Baseline	Week 12	Week 52	% Change	Baseline	Week 12	Week 52	% Change	
Cardiometabolic risk factors									
Insulin	14.3 ± 7.1	14.9 ± 6.3	14.9 ± 6.8	+4.0	18.1 ± 10.2	14.8 ± 7.1**	14.6 ± 7.8***	−19.2	<0.001
Leptin	19.0 ± 17.2	16.5 ± 14.7	20.3 ± 16.8	+6.6	23.5 ± 18.6	14.3 ± 11.1***	15.8 ± 13.6***	−32.9	<0.001
Dietary components									
Calories	1736 ± 582	1736 ± 604	1633 ± 493	−5.9	2095 ± 776	1545 ± 333***	1709 ± 497***	−18.5	0.028
% Carbs	49.5 ± 9.2	49.0 ± 7.0	49.4 ± 8.9	−0.3	54.0 ± 12.2	71.3 ± 3.5***	71.5 ± 3.2***	+32.4	<0.001
% Fat	32.5 ± 8.5	33.2 ± 6.6	32.4 ± 7.2	−0.1	28.8 ± 10.2	11.2 ± 2.0***	11.4 ± 2.8***	−60.3	<0.001
% Protein	16.7 ± 3.9	16.5 ± 4.0	17.1 ± 4.6	+2.4	16.8 ± 6.3	17.3 ± 2.5	16.5 ± 2.4	−1.7	0.501
Physiological measures									
BMI	28.5 ± 4.5	28.3 ± 4.7	28.7 ± 4.8	+0.9	32.9 ± 7.2	30.5 ± 6.6***	29.8 ± 6.8***	−9.3	<0.001
SBP	132 ± 16	126 ± 15**	125 ± 13**	−5.3	136 ± 17	122 ± 14***	127 ± 17***	−6.4	0.562
DBP	78.6 ± 10.1	77.1 ± 8.3	77.3 ± 9.3	−1.5	81.0 ± 10.1	73.0 ± 9.0***	75.5 ± 9.5***	−6.7	0.022
HDL	49.4 ± 13.0	52.0 ± 13.1**	47.9 ± 13.3	−3.0	45.5 ± 13.4	38.5 ± 9.5***	43.1 ± 10.5*	−5.2	0.497
LDL	108 ± 34	106 ± 35	108 ± 34	−0.4	112 ± 39	98 ± 32***	109 ± 33	−2.8	0.536
TCH	185 ± 43	187 ± 46	185 ± 43	−0.2	195 ± 48	170 ± 43***	185 ± 44**	−5.0	0.066
TG	144 ± 98	156 ± 138	146 ± 88	+2.0	176 ± 94	163 ± 73	163 ± 93	−7.2	0.213
EC	9.3 ± 2.9	9.5 ± 2.8	9.3 ± 2.7	−0.6	6.6 ± 2.1	8.4 ± 2.2***	9.0 ± 2.6***	+37.6	<0.001

Data are presented as mean ± SD; % change is from baseline to week 52; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to baseline by repeated-measures ANOVA; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides; EC, exercise capacity.

[a] From independent samples *t*-tests (two-tailed) of baseline to week 52 changes in program participants compared to controls.

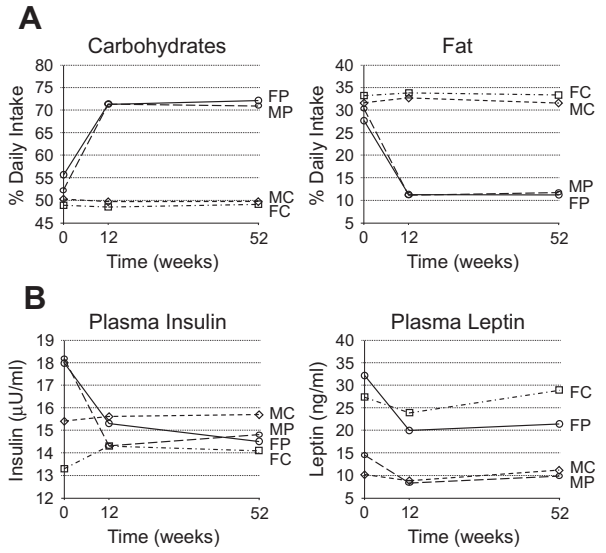

Figure 1 Changes in dietary composition (panel A) and cardiometabolic risk factors (panel B) among men and women participating in a year-long cardiovascular health program. FP, female participants; MP, male participants; FC, female controls; MC, male controls.

Effects of medications

Review of patient medical charts identified 114 prescription medications used by participants and controls at baseline. Medications ($n = 67$) known to influence circulating levels of insulin, leptin, and/or lipids as the primary intended effect, or as a secondary effect, were then partitioned into 8 categories based on function (Table 3). Separate analyses were used to assess the effects of (1) all medications in these 8 categories (composite medications), and (2) only medications influencing insulin, leptin, and lipids as a primary effect (primary medications). Because no medications were deemed to alter leptin or HDL as a primary effect, medications with the strongest secondary effects on these variables were examined.

Results of the sub-group analyses showed that composite and primary medications did not have significant effects on biomarker responses to the lifestyle change program (Table 4). Changes in insulin and leptin in participants and controls not taking medications known to influence these biomarkers or whose medication levels did not change during the study were similar to analyses encompassing all participants. Response for lipids was attenuated slightly when the effects of medications were considered. The largest effect was evident among controls, where LDL, total cholesterol, and triglyceride levels increased more in subjects with no medication changes.

Discussion

Participants who completed the year-long lifestyle change program reduced total caloric intake from >2000 calories/day to ~1700 calories/day, increased carbohydrate consumption by 30%, and decreased daily fat intake by 60%. The lifestyle intervention improved circulating levels of insulin (−19%) and leptin (−33%), which contribute to cardiometabolic risk, as well as traditional cardiovascular risk factors. Changes in circulating insulin and leptin were comparable to, or superior to, responses reported in other dietary or exercise interventions (Web Appendix), and were not significantly influenced by medication use. Men and women showed similar beneficial changes for most risk factors.

The term "cardiometabolic risk" for developing coronary atherosclerosis encompasses risk factors such as age, gender, high cholesterol, hypertension, smoking, and obesity plus additional contributing factors including insulin resistance, vascular inflammation, atherogenic dyslipidemia, and poor lifestyle behaviors. Leptin may contribute to

Table 3 Medications used by participants and controls in the cardiac lifestyle program known to affect plasma levels of insulin, leptin, and lipids.

Medication Category (n)[a]	Insulin	Leptin	HDL	LDL	TCH	TG
ACE inhibitors (13)	↓	↓	↑ ns	↓ ns	↓ ns	↓
Anticoagulants (2)						
Platelet aggregation inhibitors					↑	
Beta blockers (10)	↑	↑	↓	↓ ns	↑ ns	↑
Calcium channel blockers (9)	↑—	↓	↑—	↑—	↑—	↓—
Insulin medications (3)	▲[b]	↑[b]*				
Diuretics (6)			↓ ns	↑ ns	↑	↑
Lipid lowering medications (16)			↑[b]	▼[b]	▼[b]	▼[b]
Oral antihyperglycemics (8)						
Thiazolidinediones			↓	↑	↑	↑
Biguanides	↓	↓	↑	↓	↓	↓
Sulfonylureas	↑					

Abbreviations: HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides; ACE, angiotensin converting enzyme; ns, not statistically significant; —, effect may be neutral. Adapted from [29].
Key to medication effects: ▲ — increase, main effect; ↑ — increase, secondary effect; ▼ — decrease, main effect; ↓ — decrease, secondary effect, * — dose dependent.
[a] The number of brand name medications in each category is indicated in parentheses.
[b] Considered a primary medication in Table 4.

Table 4 Effects of medication changes on cardiometabolic risk factors and lipid measures for participants and controls in the cardiac lifestyle program.

Measures	All participants			Composite medications[a]			Primary medications[b]		
	% Change at week 52 (*n*)		Between group P^c	% Change at week 52 (*n*)		Between group P^c	% Change at week 52 (*n*)		Between group P^c
	Controls	Participants		Controls	Participants		Controls	Participants	
Cardiometabolic risk factors									
Insulin	+4.0 (75)	−19.2*** (76)	<0.001	+3.0 (61)	−16.0** (54)	0.005	+4.4 (74)	−20.3*** (75)	<0.001
Leptin	+6.6 (76)	−32.9*** (76)	<0.001	+7.9 (61)	−32.7*** (48)	<0.001	+9.1* (75)	−33.6*** (75)	<0.001
Lipid measures									
HDL	−3.0 (76)	−5.2* (76)	0.497	−2.7 (45)	−6.0* (54)	0.372	−2.8 (50)	−5.9* (60)	0.375
LDL	−0.4 (71)	−2.8 (71)	0.536	+4.3 (49)	−0.2 (58)	0.230	+4.3 (49)	−0.2 (58)	0.230
TCH	−0.2 (76)	−5.0** (76)	0.066	+2.6 (42)	−2.0 (44)	0.126	+3.1 (50)	−3.4* (60)	0.013
TG	+2.0 (76)	−7.2 (76)	0.213	+4.5 (44)	+0.8 (43)	0.798	+7.3 (50)	−4.9 (60)	0.214

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to baseline by repeated-measures ANOVA; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides.

[a] Composite medication categories are described in Table 3.

[b] Primary medication categories include: insulin and leptin — insulin medications; HDL, LDL, TCH, and TG — lipid lowering medications.

[c] From independent samples *t*-tests (two-tailed) of baseline to week 52 changes in intervention participants compared to controls.

cardiometabolic risk through atherogenic effects on the vasculature, stimulating production of reactive oxygen species and proinflammatory cytokines, which leads to oxidative stress, vascular inflammation, and atherosclerotic lesion formation [19]. Similarly, insulin stimulates the actions of various growth factors within the vasculature leading to inflammation and endothelial dysfunction [20].

Lifestyle modification can delay or prevent progression of atherosclerotic disease and significantly reduce risk of CVD mortality [21,22]. Therefore, interventions that modify both cardiovascular and metabolic risk factors may have the greatest potential to mediate cardiometabolic risk. In this study, participants in a cardiovascular lifestyle program showed significant reductions in plasma insulin and leptin, which may have beneficial anti-inflammatory and anti-oxidative effects on the vasculature.

Despite evidence that lifestyle modification can lead to significant improvements in overall cardiovascular risk profiles, gender differences in response of plasma insulin and leptin to exercise training [23] and a combination of diet and physical activity [24] have been reported. Men and women in our program showed similar reductions in plasma insulin and leptin, likely caused by changes in dietary composition and increased physical activity. These behaviors resulted in significant weight loss in both men and women over one year. Although gender differences may exist in the physiological action of insulin and leptin within the vasculature, fasting insulin and leptin are strongly correlated with percent body fat. Thus, through diet, exercise, and weight loss, both men and women may have derived similar benefit in terms of cardiometabolic risk reduction.

High consumption of fruits, vegetables, and whole grains has been associated with a favorable CVD biomarker profile, including lower fasting insulin and leptin concentrations [25]. Likewise, physical activity sustained for at least four weeks has a meaningful effect on insulin, leptin, and several other blood biomarkers implicated in CVD [26]. At baseline, program participants and controls consumed a high fat diet normally associated with obesity, insulin resistance, and atherosclerosis. Participants in the lifestyle program successfully transitioned to a low-fat diet and dramatically increased their level of physical activity, which may have been important for reducing plasma insulin and leptin levels.

One incidental benefit experienced by some participants in the cardiac lifestyle program is a reduction in the number and/or dosage of prescription medications, which has the potential to influence changes in metabolic and cardiovascular risk factors. To remove the influence of medications on risk factor response during the program, we conducted a sub-group analysis that excluded participants who changed the brand or dosage of any medication known to affect insulin, leptin, and/or lipid levels. These analyses indicate that medications did not have significant effects on biomarker response and suggest that changes in cardiometabolic risk factors during the program are primarily attributable to lifestyle changes.

Strengths and limitations

Cardiometabolic risk factors are rarely examined simultaneously in cardiac lifestyle modification programs with validated protocols and data collection methods. The prospective, longitudinal nature of this study and availability of matched controls minimized sources of bias and confounding and improved our ability to assess treatment benefits. Participants remained under the care of their primary physician, who may have prescribed changes in medications affecting plasma insulin or leptin levels. Removing pharmacological influences on risk factor modification strengthened the conclusion that participants derived meaningful metabolic and cardiovascular benefit from the program.

The Ornish Program is an established treatment alternative for CVD patients involving demanding lifestyle changes that requires motivation and significant time commitment. Baseline differences between cases and matched controls indicate that participants have

particularly atherogenic CVD risk factor profiles and would benefit most from cardiovascular risk reduction. Because careful screening is essential to identify motivated patients who would adhere to program guidelines, it was impractical to use a randomized study design. However, well-designed case–control studies are highly similar to randomized trials for estimating treatment effects [27,28]. We analyzed the data using a per-protocol approach, which included only patients who completed the program, rather than an intent-to-treat analysis. The lifestyle intervention included multiple modalities over one year, thus we were able to evaluate only short-term changes in cardiometabolic risk factors and were not able to define the relative contribution of each program component. Further, we could not assess applicability to the general public and whether results observed here are achievable outside of a controlled clinical environment. Future research will determine if improvements in cardiometabolic risk continue after participation and translate into improved clinical outcomes and develop less-rigorous cardiac interventions to maximize adherence and cardiovascular/metabolic benefit.

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Authors' contribution

LV drafted the paper and performed dietary analysis, DN managed the patient database and performed statistical analysis; DD partitioned name brand medications into functional categories, defined primary and secondary effects, and determined comparable dosages; AB and MH conducted the lifestyle intervention; FL collected dietary data and directed dietary analysis; HP collected and processed blood samples, coordinated biomarker assays; MV reviewed the paper and provided oversight as PI of ICHP; DE conceived and supervised the study and drafted the paper, which was reviewed critically by all authors.

Competing interests

The authors report no conflicts of interest with this study.

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Appendix. Supplementary data

Supplementary data related to this article can be found in the online version at doi:10.1016/j.numecd.2012.01.012.

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Feasibility of "10-Minute" Mindfulness Practice in a Therapeutic Lifestyle Change Program

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Running Title: Abbreviated Mindfulness and Lifestyle Modification

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Word Count: 5318

ABSTRACT: A potential role for mindfulness in improving diet and exercise lifestyle behaviors has been suggested. Mindfulness training usually occurs as a referral to a separate, time-intensive program, and therefore presents significant challenges. This paper describes the novel integration of abbreviated mindfulness practice into an existing therapeutic lifestyle change (TLC) curriculum within a cardiovascular risk reduction program. Ten-minute mindfulness practices (mindful eating, body scan, mindful walking, and awareness of breath, body, and sound) were incorporated into 12 weekly, 1-hour support groups. Over a 2.5 year period, 142 participants met as 30 cohorts, for a total of 335 support group sessions. Post-curriculum evaluations, follow-up questionnaires, and interviews were conducted to explore the role and impact of mindfulness practices on participants' behavior change. Mean participant rating for "meeting personal objectives and expectations" (weight loss and increased physical activity) was 4.6, on a scale of 1 (low) to 5 (high). Even with such brief exposure, 92% of respondents reported that learning basic mindfulness skills helped or helped a great deal in making the desired lifestyle changes and 32% reported using mindfulness practices in their daily lives. Abbreviated mindfulness training has the potential to add value to a TLC program.

Introduction

An essential component in the prevention of heart disease is the reduction of cardiovascular (CV) risk factors. Therapeutic lifestyle change (TLC) interventions such as nutrition, exercise, smoking cessation and stress management have shown improvements in modifiable CV risk factors [1-5]. Yet, in spite of increased general knowledge about the benefits of healthy eating and regular exercise, only 23% of American adults consume 5 or more fruits and vegetables per day [6], 34% maintain a normal weight [6], and less than 5% participate in the recommended moderate-intensity physical activity [7]. Given the difficulty in making and maintaining lifestyle changes, the authors wanted to explore simple and practical techniques that might be integrated into a CV lifestyle change program as well as its participants' busy lives. The purpose of this report is to describe the integration of abbreviated mindfulness practices into a TLC program and assess its feasibility, using data collected from both a follow-up questionnaire and participant interviews.

Background

Mindfulness is moment-to-moment nonjudgmental awareness that can be cultivated by deliberately paying attention to things we ordinarily don't give much notice. Rooted in ancient Eastern traditions, mindfulness training was introduced into modern Western society and healthcare in 1979 when Jon Kabat-Zinn developed and first offered "Mindfulness-Based Stress Reduction" (MBSR) for the management of stress, anxiety and chronic pain [8]. MBSR is a well-defined and researched psychoeducational program with intensive training in mindfulness meditation. One of the challenges of mindfulness training is that the curriculum is generally

long and includes 8 weekly 2-3 hour group sessions, 30-45 minutes of daily meditation practice between classes, and an all-day retreat.

A. How might mindfulness support our participants in adopting healthier lifestyle behaviors?

Mindfulness is awareness of the present moment, without criticism or judgment. Paying attention to the present moment is a simple act with profound consequences. Our lives unfold in each moment; we make choices about what to eat or not eat in each moment; we decide whether to go for a walk or sit on the couch in a moment; we notice that we are satisfied enough to get up from the table in a moment. If we are not aware of our moments, we miss opportunities to make better choices for ourselves. When we are aware of these moments of choice, we increase our potential to control and regulate our impulses. Anything that improves self-regulation will be helpful in changing behavior [9,10].

Mindfulness is awareness of the present moment*, without criticism or judgment.* When we approach each of life's moments without criticism or judgment, we cultivate compassion for ourselves and others. This kindness serves us well every time we experience a setback in our efforts to replace unhealthy lifestyle habits with new healthier habits. When critical or judgmental thoughts do occur, we are apt to notice them sooner, reducing the time spent in unproductive and confidence-eroding negative "self-talk". In this kind and compassionate environment, self-efficacy has the potential to flourish [11].

B. How is the skill of mindfulness developed?

All of us have the capacity to be mindful and to cultivate the ability to pay attention in each moment. Mindfulness grows in the presence of seven attitudinal factors that are all interconnected: 1) Beginner's Mind (seeing things as they really are, as if for the first time); 2) Patience (realizing that things unfold in their own time); 3) Non-judging (becoming a less reactive and more impartial witness to our thoughts, feelings, and experience); 4) Non-striving (having no goal other than to be oneself); 5) Acceptance (being willing to accept things as they already are, in the present); 6) Letting Go (becoming less attached to our thoughts, feelings, and experiences), and; 7) Trust (looking inside oneself for guidance). In the standard MBSR curriculum there are ample opportunities to nurture these qualities in formal meditation practices like the body scan; sitting with an awareness of breath, sensations, and thoughts, and; bringing mindful attention to eating, walking, and movement [8].

C. Formal and informal mindfulness practice

Cultivating these 7 attitudinal factors with formal mindfulness practices can lead to the more "informal" and practical application in everyday diet and exercise behaviors. When we eat mindfully, beginner's mind allows us to fully taste and savor each bite of food, to consider tasting something outside our usual habit, to notice when we are full and when we are hungry, and to notice how this food affects us. When our mind wanders during meditation, patience and a non-judging attitude enable us to return kindly (again and again) to our focus. When we walk mindfully—not for exercise, not to get anywhere—we are nurturing our ability to simply connect with and be more aware of our body, and to do something good for ourselves without striving for a particular outcome. When we scan the body and find discomfort or pain, we

practice meeting it with an acceptance that this is just how things are in this moment and hopefully are less likely to resist what is already there. As all these qualities develop and interact, we gain greater insight into ourselves, our habits, and our moment-to-moment experience. We develop a different relationship with ourselves, our bodies, and the inevitable setbacks that occur as we attempt to change our habits. When we accept that setbacks (like the distractions in meditation) are inevitable, we are less critical of ourselves when they do occur. We are also able to view them more as temporary distractions, let them go, and return to our plan. Importantly, we are able to do this with less emotional reaction and with more kindness toward ourselves. We grow in wisdom, confidence, trust in ourselves and our inner resources, and ultimately our ability to change our behavior.

Review of Literature

A review of the literature provided evidence that mindfulness has been effectively used to modify behaviors such as anger [12], addictions [13], eating disorders [14, 15], and attention deficit disorder [16] and to prevent relapse of depression [17]. From the fields of medicine, nursing, and psychology, Proulx [18] reviewed 20 peer-reviewed clinical studies on MBSR interventions between 1982 and 2003. Among several recommendations, the author encourages the application and study of MBSR in health promotion, since mindfulness seems to lead to improved self-regulation and more adaptive coping. A growing interest in applying mindfulness to making healthy lifestyle behavior changes is evident in recent studies and commentary in the areas of weight loss [19], exercise [20], and smoking cessation [21], with Dutton [20] recommending that a referral to a mindfulness program would be beneficial for those wanting to change lifestyle behaviors.

Within the field of CV risk reduction, Edelman et al [22] evaluated a personalized health plan (PHP) which included a significant mindfulness component, in a 10-month trial with 154 subjects, randomly assigned to the PHP or usual care. The authors reported a modest but statistically significant improvement in the Framingham Risk Score (FRS) from baseline to 10 months (16% in the PHP arm vs. 12% in the usual care arm). Low dose MBSR (MBSR-ld) was evaluated with 48 healthy working adults in a university workplace setting [23] who were randomly assigned to the MBSR-ld intervention or a wait-list control group. The mindfulness intervention consisted of 1-hour lunch time group meetings for 6 weeks and 20 minute daily practice (at work, on workdays only) using an instructional CD. Data showed a significant decrease in perceived stress and a significant improvement in subjective sleep quality, while increasing daily mindfulness. The author concluded that low dose workplace MBSR is an effective adaptation of the MBSR approach for healthy working individuals limited by time.

With evidence that MBSR can be helpful in a variety of settings, that the MBSR curriculum can be incorporated into an existing clinical intervention, and that a lower dose of MBSR can be effective, we hypothesized that brief exposure to mindfulness in a TLC intervention for adults with CV risk factors could be beneficial. To our knowledge, this is the first report to describe the integration of "10 minute" mindfulness training in a CV risk reduction program.

Methods

Population, Setting and the TLC Intervention

A TLC intervention targeting military healthcare beneficiaries ($\geq$ 18 years of age) with at least two CV risk factors and evidence of subclinical atherosclerosis was conducted over a 2.5 year period from January 2008 to July 2010 at Walter Reed Army Medical Center in Washington

DC. Figure 1 summarizes the program. In the first 2 weeks of the TLC intervention, each participant received 6 hours each of nutrition and exercise group instruction from a dietitian and health fitness instructor respectively. During the nutrition classes, participants received instruction on components of the Mediterranean diet, label reading, portion control, and food intake monitoring. Exercise instruction was both didactic and monitored, consisting of basic exercise principles (frequency, intensity, type and time), equipment use, safety, and heart rate monitoring. These 2 weeks of classes were then followed by 12 one-hour weekly support group meetings facilitated by a nurse experienced in teaching MBSR. The goal of the weekly group sessions was to provide ongoing support to participants as they began to make changes in their diet and exercise behaviors. The weekly support groups included 4 components: 1) a 20 minute discussion to share eating and exercise successes, challenges, and self-discovery; 2) a 10 minute discussion of individualized nutrition and exercise goals for the coming week followed by writing of those goals 3) a 20 minute presentation of an informational topic (from the established curriculum) supporting the process of making change, and; 4) a 10 minute mindfulness practice and/or discussion.

Mindfulness Integration

Over the 12 week intervention, a total of 2 hours of actual mindfulness instruction (practice or discussion) took place. Written handouts augmented in-class practices, but home practice was not required. Every attempt was made to correlate a mindfulness practice with the weekly curriculum topic or theme (Table I). For example, the discussion of habits and automatic behavior was paired with a mindful eating exercise that directs participants to use all the senses to notice a food's taste, texture, color, and smell---—discouraging mindless automatic eating.

The discussion of how beliefs and thoughts might affect one's behavior was paired with a short sitting meditation focusing on the breath. During this simple breath awareness practice, one typically begins to notice how much automatic thinking is going on, and how the thought makes one feel. With regular practice, awareness of the mind's automatic habits and patterns of thinking (i.e. self-criticism and judgment) emerge.

Initial program evaluation

Of the 142 program completers, 87% provided an anonymous course evaluation at the final group session, with feedback on teaching methods, presentation style, course content, and overall satisfaction. On a scale of 1 (low) to 5 (high), participants' mean overall rating for "satisfying personal expectations" was 4.6. In response to open-ended questions about what was most--and least—helpful about the support groups, twice as many respondents commented that the mindfulness practices were helpful. When asked to "List 3 specific things you are doing differently as a result of your participation in this study", 32% indicated they had incorporated some form of mindfulness practice into their daily lives. Based on these encouraging findings, the authors conducted a more formal retrospective evaluation of the TLC program to assess effectiveness of individual components and elicit suggestions to improve the intervention. Complete main study findings will be reported elsewhere. This report focuses specifically on the feasibility and effectiveness of including a mindfulness component in future support groups.

Formative Evaluation Methodology

This formative evaluation was designed to assess the participants' experiences with the overall TLC intervention and its separate components with the intent of improving future

programs. For the mindfulness component being reported here, the objective of the evaluation was to assess the feasibility of the abbreviated mindfulness integration, answering the following specific questions: 1) Is it realistic to teach mindfulness skills in such an integrated and abbreviated way; 2) Will the participants be open and receptive to learning basic mindfulness skills in the context of a CV risk reduction program; 3) Will participants be able to learn basic mindfulness skills taught in this way, and; 4) Will participants find the mindfulness skills useful in improving their diet and exercise habits.

The formative evaluation consisted of two components: a 26-item questionnaire (Table II) and a structured telephone interview (Table III). Both instruments were developed by the authors and reviewed for content validity by 2 professionals with experience and skills in qualitative research methods. Approval was obtained from the Institutional Review Board of the Walter Reed Army Medical Center (Washington, DC) prior to contacting 140 of the 142 program completers and the 21 non-completers who agreed to be contacted for follow-up. The questionnaire was mailed to all program completers. The opportunity to participate in a 20-30 minute phone interview was offered to those enrolled in the final year of the program (n=70) and all program non-completers (n= 21). Consenting respondents were identified by study identification number only and were asked to return the questionnaire and/or telephone interview consent in the enclosed self-addressed stamped envelope.

The decision to limit interviews to those participating in the last year of the program was based on two assumptions: 1) these participants would be closer in time to their actual experience with presumably better recall, and; 2) during the second year of the program minor curriculum modifications were solidly in place and being consistently applied. However, all non-

completers were contacted to hear the full extent of their experiences. To enhance reliability of the interview data, all interviews were conducted by a single trained interviewer who was familiar with the program, but had not been involved in program delivery. All interviews were tape recorded with the participants' consent, then transcribed verbatim by the primary author. For the phone interviews, qualitative data were collected using a standardized open-ended interview format [24]. The benefit of this method is that each respondent is asked the same questions, in the same order, and in the same way. A structured interview guide kept the interview focused and standardized while also allowing participants to describe what had been meaningful and salient for them in their experience of changing diet and exercise behavior.

Data Analysis Plan

Since the initial program evaluation and the follow-up questionnaire asked both fixed-choice and open-ended questions, a combination of quantitative and qualitative data are presented. The quantitative data were analyzed with Microsoft Office Excel 2007 (Redmond, Washington) and SPSS Software (Version 14.0, SPSS Inc., Chicago, IL). Subject characteristic variables were summarized descriptively. Continuous variables were expressed in terms of descriptive statistics including the mean and range. Categorical variables were summarized in terms of frequencies and percentages. All responses to both the questionnaire's open-ended questions and the verbatim transcripts of the interviews were reviewed independently by the authors to identify preliminary themes or patterns. Following consensus discussions and further reviews by all authors, categories were finalized.

Results

Demographics

In 335 support group sessions (30 cohorts) conducted over a 2.5 year period, 142 adults completed the TLC intervention program with the goal of improving their diet and exercise habits. All were military healthcare beneficiaries: 20% active duty, 38% retired, 41% dependents, 1% other, 64% female, and with a mean age of 55 years (range 26-78 years). Racially, 47% were White, 45% African American, and 8% other. For a demographic comparison of the responding groups to the original participant group, see Table IV.

Follow-Up Questionnaire

Of the 142 program completers, 140 consented to be contacted for follow-up. Sixty-nine of the 140 (49%) completed and returned the questionnaire. In response to the open-ended question, "Besides diet and exercise, what OTHER information or skills most helped you make lifestyle changes?" 47% of respondents mentioned "mindfulness" and responses were categorized as follows: 33% relaxation, 19% breathing/meditation, 19% stress management, 10% more positive attitude, 7% self-compassion, 7% coping skills and 5% mindful eating. In response to what "dietary information or skills were most helpful", mindful eating was specifically mentioned by 3 participants. When asked to specifically rate the value of "learning basic mindfulness skills", 92% said that it "helped" or "helped a great deal".

The questionnaires were completed 6 months after the last cohort support group met. Therefore, for the earlier cohorts, the questionnaires were being completed almost 3 years after their active involvement in the program (Figure 2). Even so, respondents rated their current satisfaction with efforts to be more mindful and aware as 3.2, on a scale of 1 (low) to 4

(high), and most often cited using the following mindfulness practices (frequency of response): mindful eating (36), relaxation/meditation/prayer (29), awareness (14), breath (12), and a more positive attitude (7).

Interviews and Main Themes

Of the 91 participants who were offered the opportunity to participate in a 20-30 minute phone interview, 41 (45%) consented, including 4 program non-completers. A total of 35 interviews were conducted of which 3 were program non-completers. Six consenting participants were unreachable after several attempts and therefore not interviewed. In the interviews, 69% described the mindfulness practices as helpful, 14% as neutral, 9% as negative, and 9% had either an incomplete interview or left the program too early to be able to comment on the mindfulness experience. Although most respondents gave overwhelmingly positive comments regarding the TLC program design and its implementation, 9% suggested that more of the support group time be dedicated to the mindfulness practices. Using the study's 4 feasibility questions as the organizing framework, participants expressed their personal experiences with the mindfulness practices as follows:

Question 1. Is it realistic to teach mindfulness skills in such an integrated and abbreviated way?

Three interviewees described their satisfaction in these words: "... the little tidbits along the way were really helpful"; "...I think they did it very well"; and from a participant who went on to take the [standard curriculum] MBSR course after completing the TLC intervention "...actually, I would say, generally speaking, I got just as much out of this program as I got out of the whole [MBSR] class...the whole [MBSR] class was more meditation oriented than mindfulness, in a

sense." Three participants commented that they would have liked more of the mindfulness practice: "...I felt we didn't spend enough time on mindfulness...very short things...I wished that could have been expanded..."; "...focus [more] on some of those stress management techniques...it's worth the time to spend really getting down to the details, controlling your breathing, taking a minute before you eat to think about what you're doing"; and " ...the whole thing could have been extended."

Question 2. Will participants be open and receptive to learning basic mindfulness skills in the context of a CV risk reduction program?

Eighty-three percent of the participants interviewed were receptive to the mindfulness practices and expressed positive comments about its usefulness versus 17% (n=6) who gave responses that would be considered unreceptive. Two of the 6 described not needing the mindfulness practices: "...probably the least important part of it for me...I have learned a lot about relaxation techniques...and meditation...and listening to my own sense of spirituality...I kind of was where I wanted to be already on that I think...it was the thing I needed the least of"; and "I think my mind was already made up, sort of...how I deal with stuff...I'm pretty stress-free...there wasn't a lot of benefit for that part of the program because I feel I've got my brain under control most of the time." Three of the 6 were skeptical of its usefulness: "...I didn't find those as helpful as the more concrete aspects of the nutrition and exercise...I kind of just sat through that stuff...I took away a general understanding about how that aspect affects health but didn't take away from it any practices that I continue to use"; ..."it's more an alternative medicine style that if you're more facts-based and science-based...you had to really reach to be supportive of the concept..."; and lastly, "...instruction was good...it just isn't my thing". This

final comment is from a participant who found the mindfulness practices extremely challenging: "...I hated all of them. I really did...the mindful eating just doesn't work with me...I just can't focus like that...I don't like the concentrating on breathing in and out...I just had a very hard time with all of that...I just basically didn't do it...it's just counter to my personality I guess...so I didn't get a whole lot out of that...it's hard for people like me to sit still long enough and to relax."

Question 3. Will participants be able to learn basic mindfulness skills taught in this way?

The best evidence that some participants indeed learned basic mindfulness skills are these 9 examples of using mindfulness skills in times of need: "...In the last 3 or 4 months I have had a lot of stress in my life...and I was able to sort of pull that back and remind myself 'ok well now I need to do some of these mindfulness practices...I can deal with this in a productive way' "; "...If I'm not able to eat right or exercise, I can start again tomorrow. I still use that word of encouragement"; "...I just have a better outlook...knowing that I can take control and do certain things...I start to employ the techniques I've learned..."; "...after I finished with the program...I lost my daughter due to a car accident—it was very stressful for me...I got off track a little while...I started going back to it...doing a lot of the stress techniques that they had showed us...went back to the food program...on my own...joined a weight loss support group, and just by doing that, by using the notebook, writing down all my food and everything, I've actually lost 67 pounds...so I did very well"; (from a couple who completed the program together) "...mindful eating...made a very good impression...we sit down to dinner, the two of us, and you know, right in the middle of digging in, it's like wait a minute, wo, wo, wo...stop, inhale, relax, enjoy"; [Mindfulness] "...that was good for me...those nights that my mind is just racing...I

can incorporate some of that deep breathing...during the day when I feel like I might be getting out of control with my thoughts--because I've been exposed to that in those sessions--I watch my breathing...that's helpful to me in that way"; ..."made me look at life a little bit different...that was a great eye-opener for me...still plays a very important part in my life"; "...very helpful...still is...I often take 5 or so minutes...kind of turn away from my desk and look out my window and go to my breathing and you know, I find that to be very calming, and very, very relaxing...leave for a moment and then come back with a little bit fresher outlook"; "...I was actually able to learn how to focus well enough to bring my blood pressure down when I needed to...still use it now when I find myself under a lot stress...things like deep breathing, and focusing, and just relaxing".

Question 4. Will participants find the mindfulness skills useful in improving their diet and exercise habits?

These 6 participants unequivocally describe the mindfulness practices as contributing to their success in improving their diet and exercise behaviors: "I was trying to do something that I had tried a million times before to do and I hadn't been able to do...[before] it was either all on or all off...it was very unforgiving...[here] I learned...forgive yourself...then just go on, which is so much more productive than this thing of binging, gorging...I feel so lucky...it has changed my life"; [the mindfulness practice] "...that was golden...one of the biggest issues I have is being aware of when I'm eating, and being aware of what I'm eating, and being aware that...food is not always the answer to stress, but there are other alternatives to stressful situations...that was...that is, a tool that I use very often, and so, that was the best gift I got from the whole thing...mindful awareness is critical"; "...mindfulness is an attempt to realize or see that there's

always something changing, always something different, and that's a good thing...I thought that was a very important thing...mindfulness is the biggest help in doing this whole thing"; [the facilitator] "was non-judgmental about people...probably the biggest thing that people need, that they have so little of these days is acceptance...that was very good..."; "...slowing down while eating...enjoy what you're eating...get away from the sort of, hit-and-run, shove-it-down...way of approaching meals"; "...mindfulness-based stress reduction... I thought that was a very good thing...that whole calming inner presence made it possible for me to enjoy my meals, my time, my life in general...helpful...later...as I went to combat."

Discussion

By design, the mindfulness skill practice in this study was limited to 10 minutes. The practical aim was to introduce mindfulness so that participants might develop beginning skills without placing greater demands on their time. At enrollment, participants were unaware that mindfulness training would be included in the support group curriculum. Therefore, in respect to the mindfulness component under evaluation, they were not actively choosing to learn mindfulness and could be considered a reasonably random or unbiased sample. The vast majority of respondents reported a positive experience and described the experience as helpful as they attempted to change their diet and exercise behaviors. The value of the experience can be heard in phrases like "because I've been exposed", "helped me realize", "eye-opening", "skills I fall back on", "a tool I use very often", "best gift I got", and "I feel so lucky... it has changed my life". While the time spent in formal mindfulness practices was indeed limited, mindfulness was the underlying thread within the support group sessions. In the words of one participant ..."it was like the whole program was about learning mindfulness". It was

encouraging that so many participants benefitted and continue to use the skills they learned, however, it is a limitation that this evaluation lacks feedback from that half of participants who did not respond to the questionnaire or interview.

Although most respondents felt the "10-minute" mindfulness practice was a positive experience, there were a few participants who found it challenging to maintain focus on the meditative object (food, breath, sound, body sensations). There were a few who felt that the mindfulness practices were just "not right" for them and a few who were skeptical, and either unwilling or reluctant to try. However, in the primary author's experience, these reactions are typically seen even in the longer standard MBSR curriculum. Facilitators should be aware that there will always be at least a few participants who are skeptical, or who find the practices particularly challenging, and remember to proactively raise these possibilities to encourage discussion within the group.

This study has demonstrated the feasibility of integrating abbreviated mindfulness training in a TLC program for patients with CV disease risk factors. There is promising data suggesting that mindfulness-based interventions are effective. Research in this area is beginning to investigate the mechanisms of action underlying mindfulness-based interventions. Shapiro and colleagues [25, 26] suggest a model where the fundamental building blocks of mindfulness (intention, attention, and attitude) are interconnected aspects of a process occurring simultaneously. In this model, the three core components of mindfulness: 1) intention (why one is practicing), 2) attention (observing one's moment-to-moment experience), and 3) attitude (how we pay attention) result in a significant shift in perspective, called "reperceiving" or changing one's relationship to thought rather than attempting to alter the thought itself. They

theorize that reperceiving is at the heart of the change and transformation affected by mindfulness practice, thus allowing an individual to become less controlled by arising emotions and thoughts and then less likely to automatically follow them with habitual reactive patterns. A more accepting, compassionate, and systemic approach is then brought to self-regulation and health. "How" we pay attention has been variously called "with kindness", "without judgment", "without criticism", or "self-compassion".

Self-compassion emerged as an important theme in this study as some participants described learning to accept and forgive themselves. In one participant's words, "This has been one of the best lifestyle classes I have ever attended. The fact is...you do not have to beat up on yourself." Neff [27] proposed that self-compassion entails being kind and understanding toward oneself in instances of pain or failure rather than being harshly self-critical; perceiving one's experiences as part of the larger human experience rather than seeing them as isolating, and; holding painful thoughts and feelings in mindful awareness rather than over-identifying with them. Self-compassion has since been studied in the context of mindfulness and stress reduction when Shapiro et al [28] found that healthcare providers in a 6-week MBSR course showed a significant increase in self-compassion scores (SCS), and that self-compassion appeared to mediate reductions in stress. Neff [29] explored self-compassion and academic achievement in college students, finding that self-compassion was positively associated with mastery goals and more adaptive ways of coping with failure. Self-compassion scores are also strongly indicative of psychological health [30], with lower SCS associated with self-criticism, depression, anxiety, rumination, thought suppression, and neurotic perfectionism, and higher SCS associated with life-satisfaction, social connectedness, and emotional intelligence. In a pilot

study of "compassionate mind training" with 6 participants chronically incapacitated by shame and self-criticism, Gilbert [31] reported significant reductions in depression, anxiety, self-criticism, shame, inferiority and submissive behavior. There was also a significant increase in the participants' ability to be self-soothing and focus on feelings of warmth and reassurance for the self. Although directly teaching compassion is not part of mindfulness training for depression relapse [17], compassion is believed to emerge naturally from its practice. Some of our participants reported being less critical of themselves, feeling kinder toward themselves, being grateful for the concern and help from group members, and motivated by the fact that they were not alone in struggling to make difficult changes.

Besides self-compassion, another potential mechanism of action may be the participants' relationship to thoughts. For example, in mindfulness-based cognitive therapy (MBCT) for the prevention of depression relapse [17], individuals are taught to recognize their thoughts and feelings with a non-judgmental attitude, and then interrupt, let go of, or step out of the unhealthy cycle of thought. Similarly, in a pilot study [15] of mindfulness-based eating awareness training (MB-EAT), Kristeller observed that mindfulness training can interrupt the usual relationship between thoughts, emotions, and behavior. After further exploration and study, Kristeller et al [32] concluded that "acceptance-based" methods, like MBSR, may offer an approach that is more effective than cognitive behavioral therapy alone, in internalizing and maintaining change. The experience of our respondents would suggest that even "10- minute" exposure to mindfulness with a trained MBSR facilitator has benefits in the areas of awareness, self-regulation, self-acceptance, and self-efficacy. Mindfulness may well provide an important

bridge between diet and exercise knowledge and actual behavior change, as succinctly stated in the words of one participant -- "the integration of awareness and practice".

Conclusions

"10-minute" mindfulness training was successfully integrated into an existing TLC intervention curriculum in a CV risk reduction program. With very few exceptions, participants demonstrated a willingness to engage in mindfulness practices during the weekly support group meetings. In a program evaluation conducted at each cohort's final group meeting, and in a formative evaluation conducted 6 months after the program ended, most participants credited mindfulness training with contributing to their success in making positive changes in their diet and exercise behaviors. Even with such limited exposure and training, many participants continued to include mindfulness practices in their daily lives.

To answer the original feasibility questions: 1) It was realistic to teach short, introductory mindfulness practices; 2) Most participants were receptive; 3) Receptive participants did learn basic mindfulness skills, and; 4) Mindfulness may have helped participants make lifestyle changes. In conclusion, it is feasible to include "10-minute" mindfulness training in future CV risk reduction programs. Randomized studies are needed to explore the utility of "10-minute" introductory mindfulness training on primary outcomes in TLC intervention programs.

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Figure Legends:

Figure 1. TLC Flow Chart

Figure 2. Elapsed time from program completion to return of questionnaire

Figure 1

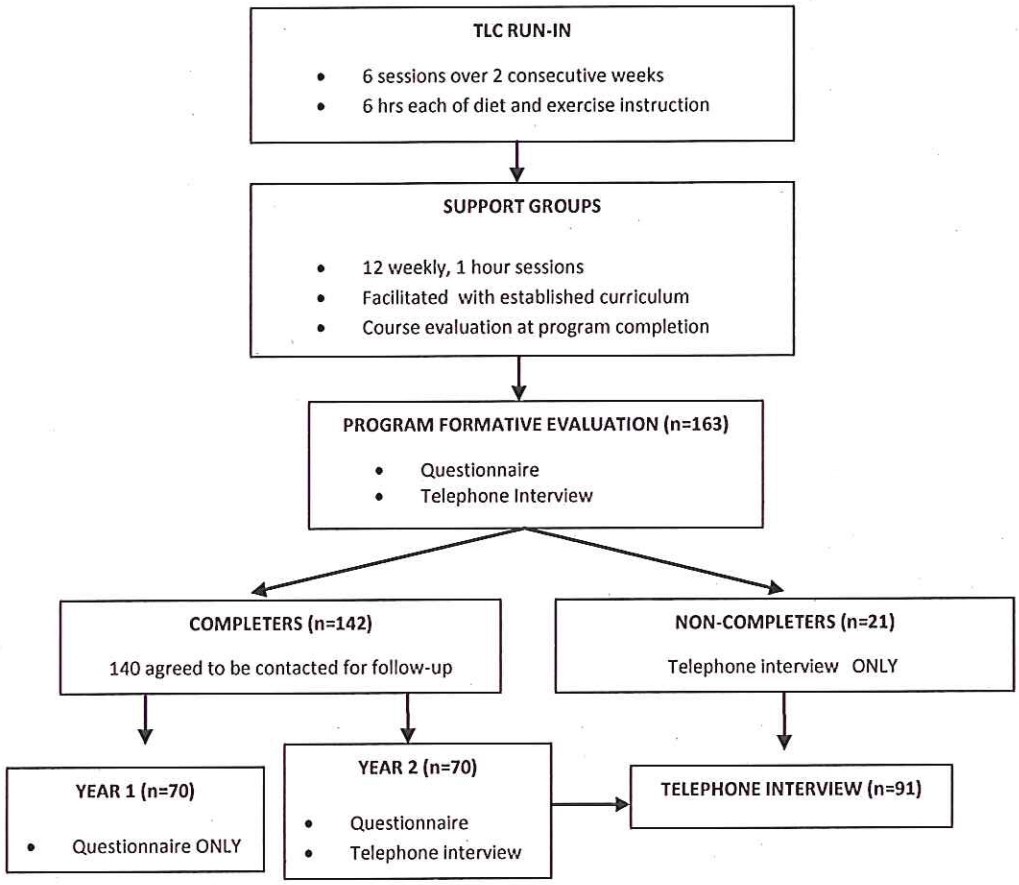
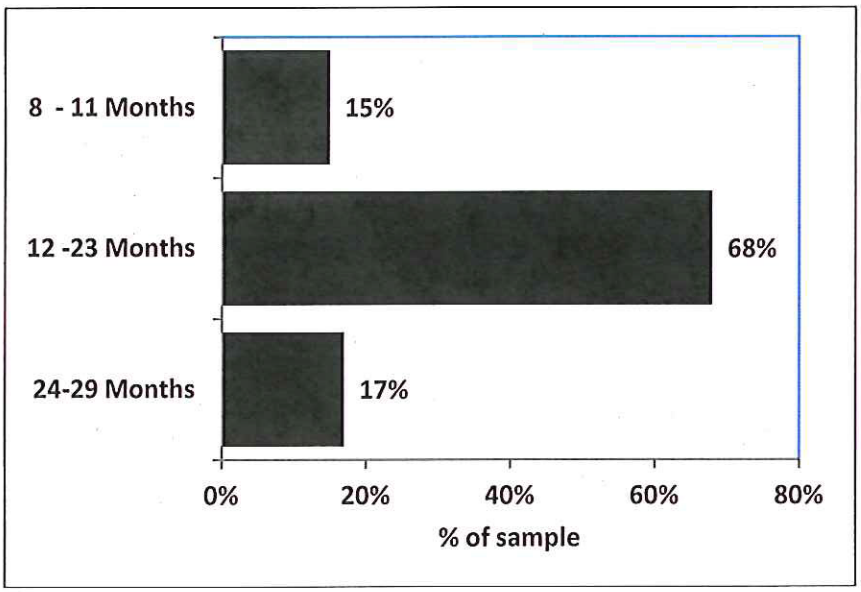

TLC RUN-IN
6 sessions over 2 consecutive weeks
6 hrs each of diet and exercise instruction
SUPPORT GROUPS
12 weekly, 1 hour sessions
Facilitated with established curriculum
Course evaluation at program completion
PROGRAM FORMATIVE EVALUATION (n=163)
Questionnaire
Telephone Interview
COMPLETERS (n=142)
140 agreed to be contacted for follow-up
NON-COMPLETERS (n=21)
Telephone interview ONLY
YEAR 1 (n=70)
Questionnaire ONLY
YEAR 2 (n=70)
Questionnaire
Telephone interview
TELEPHONE INTERVIEW (n=91)

Figure 2


8 - 11 Months
15%
12 -23 Months
68%
24-29 Months
17%
0%
20%
40%
60%
80%
% of sample

Tables

TABLE I. Session overview of 12-week Support Group Curriculum	
INFORMATIONAL TOPIC	**MINDFULNESS PRACTICES and DISCUSSION POINTS**
1. Habits Triggered automatic behaviors	***Mindful Eating***: Bean tasting Break out of old eating patterns. Savor food. Notice early signs of satiety. Consider being satisfied with less. ***Mindfulness Principles***: Beginner's Mind, Non-judging
2. Health Beliefs Beliefs →thoughts → mood → behavior	***Mindful Practice***: Awareness of Breath Notice how much thinking goes on; reactions (critical of self, reacting to thought itself); It is possible to train our minds. ***Mindfulness Principles***: Patience, Beginner's Mind, Non-judging
3. Ready, Willing, and Able Motivation, self-regulation, self-efficacy	***Mindful Exploration:*** The 9 Dots Exercise When we see the 9 Dots as a box we are unable to solve the problem; this limits our solutions. ***Mindfulness Principles:*** Patience, Beginner's Mind, Non-judging
4. Goal Setting Best goals are: written, clear and simple, an achievable stretch	***Mindfulness Practice:*** Body Scan Body awareness: observe sensations w/o trying to change them; train the mind to let go of one, move to next ***Mindfulness Principles:*** Acceptance, Letting Go, Beginner's Mind, Non-judging
5. Social Support Social support increases success with change	***Mindfulness Practice:*** Loving Kindness or Compassion Meditation Value connection to self and others. Treat self as a friend. Consider giving difficult people another chance. ***Mindfulness Principles:*** Beginner's Mind, Non-judging, Acceptance
6. Nutrition Review Healthy fats, fiber, portion control. Stuck somewhere?	***Mindful Eating:*** Grain tasting (with less instruction this time) Re-assess and recognize progress; identify current food challenges; re-commit to goals. ***Mindfulness Principles:*** Beginner's Mind, Non-judging, Trust in Self
7. Managing Time Tools: planning, prioritizing, streamlining processes, beware of perfectionism.	***Mindful Discussion:*** Our relationship with time Time is a product of thought. Live more in the present. Take time to just "be". Simplify our lives. ***Mindful Demonstration:*** Covey's Golf Balls + Jar
8. Exercise Review Goals, successes, stuck somewhere? Plan to maintain habits after program completion.	***Mindful Walking:*** Walking to develop awareness, not to get somewhere. Being in touch with body prevents injury, maximizes benefits of exercise. ***Mindfulness Principles:*** Non-striving, Acceptance, Trust in Self
9. Thinking Can Be Hazardous to Health Pessimism, rumination, depression, anxiety, hostility, vs optimism, gratitude, compassion.	***Mindful Exercise:*** "I am grateful for..." Take time to notice what is good in our lives. Notice there is often more right with us than wrong with us. Appreciate how others help us. ***Mindfulness Principle:*** Beginner's Mind, Acceptance, Non-judging
10. Stress Management Effects of stress; coping (relaxation, problem-focused coping, emotion-focused coping)	***Mindful Discussion:*** Impact of mindfulness on stress reaction cycle: less emotional appraisal of events; break cycle of automatic reactions; acceptance of things "as they are" reduces additional suffering; enhanced emotion-focused coping (learning to live with "what is").
11. Stress at Work Personality traits, burnout, sleep, coping	***Mindful Discussion:*** Finding meaning in work, or finding new work ***Mindfulness Principles:*** Beginner's Mind, Acceptance, Letting Go, Trust in Self
12. Transition Celebrate successes, review resources and plan	***Mindful Eating:*** Shared Healthy meal ***Mindfulness Principle:*** Trust in Self

Table II. Follow-Up Questionnaire	
QUESTIONS	
Q1. I decided to volunteer for the lifestyle modification program because...	8 choices + "other"
Q2. During the program, how satisfied were you with your dietary efforts?	1 = very dissatisfied 2 = dissatisfied 3 = satisfied 4 = very satisfied + comments
Q3. How satisfied were you with your dietary accomplishments?	
Q4. During the program, how satisfied were you with your exercise efforts?	
Q5. How satisfied were you with your exercise accomplishments?	
Q6. Overall, how satisfied were you with what the program provided?	
Q7. From the following list, check all the dietary obstacles that you faced...	11 choices + "other"
Q8. From the following list, check all the exercise obstacles that you faced...	7 choices + "other"
Q9. What dietary information or skills were most helpful for you?	Open-ended, with space for 3 items
Q10. What exercise information or skills were most helpful for you?	
Q11. Besides diet and exercise, what other information or skills most helped you make lifestyle changes?*	
Q12. Please rate how useful each of the following support elements was for you, from a list of 12 support elements, including **"learning basic mindfulness skills*)**	1 = made things worse 2 = did not help 3 = helped 4 = helped a great deal
Please comment on the following elements of the program structure: Q13. Length of the nutrition classes... Q14. Length of the exercise training... **Q15. Length of each weekly support group meeting*** Q16. 2-week initial training **Q17. Total of 12 weekly group meetings***	Too short Too long Just about right
Q18. If a friend were in need of similar help, how likely would you be to recommend a program like this?	1 = definitely would not 2 = unlikely 3 = likely 4 = definitely would
Q19. If you were to seek help again, how likely would you be to come back to a program like this?	
Q20. Is there anything else you would like to add about your participation in the program?*	Open-ended
Q21. How satisfied are you now with your efforts to maintain your healthier dietary habits?	1 = very dissatisfied 2 = dissatisfied 3 = satisfied 4 = very satisfied + comments
Q22. How satisfied are you now with your efforts to exercise regularly?	
Q23.How satisfied are you now with your efforts to be more mindful and aware?*	
Q24. What positive dietary habits have you been able to maintain?	Open-ended, with space for 3 items
Q25. What positive exercise habits have you been able to maintain?	
Q26. What mindfulness practices have you been able to maintain?*	
***items either asking directly about experience with mindfulness or presenting opportunity to describe it** Each question also provided the option to add comments	

Table III. Interview Guide

Introduction

"Hello...my name is... and I'm calling from... You have recently given us permission to call and talk with you about your experiences in our lifestyle change program. This interview should take about 30 minutes. And I'd like to remind you at this time that I will be tape recording the interview. Is this a good time for you to talk?" (Continue or reschedule for another time)...

"We are trying to learn more about your experience with the lifestyle change program."

"As you recall, your efforts in the program occurred in 2 phases: the 2-week Run-In where you attended 6 diet and 6 exercise classes, and 12 weekly Support Group meetings, where you learned additional information about making change, received group support, and were introduced to mindfulness for stress reduction "

"We want to know both what helped and what didn't help you. Please answer the following questions. Your responses will be used to improve our future programs. "

Questions

1. Why did you decide to participate in the lifestyle modification program?

2. Please describe any goals you had in mind.

3. How had you tried to accomplish these goals in the past?

4. In general, please describe your overall experience with the program.

   Probes: What did you like about the program? What did you dislike about the program?

5. Please describe any difficulties you experienced in participating in the program.

   Probe: What, if anything, would have made it easier for you to participate in the program?

6. Can you describe any ways that the program has made a difference in your life?

   Probe: Please describe any changes you made as a result of participating in the program.

7. Now, I'd like to ask you about each of the 4 main aspects of the program: nutrition, exercise, group support, and mindfulness for stress reduction.

   a. what was your experience participating in the nutrition education?
   b. what was your experience participating in the exercise instruction?
   c. what was your experience meeting in your weekly group?
   d. what was your experience with the mindfulness practices?

8. Can you describe any obstacles you've faced since you left the program?

9. What suggestions do you have for ways to improve the program?

10. And finally, are there any other comments or suggestions you would like to share?

Table IV. Demographics			
	Completers n=142	Survey Respondents n=69	Interviewees n=35
Age - years	55 (26-78)	57 (30-78)	58 (39-75)
Female	64%	72%	63%
Race			
Caucasian	47%	57%	66%
African American	45%	39%	28%
Other	8%	4%	6%
Military Status			
Active Duty	20%	20%	20%
Retired	38%	29%	34%
Dependent	41%	48%	46%
Other	1%	3%	0%

The Importance of Weight Loss for Effecting Molecular Change during Intensive Cardiovascular Risk Reduction

Ellsworth DL, Croft DT Jr, Burke A, Haberkorn MJ, Patney HL, Mamula KA, Vernalis MN. Windber Research Institute, Windber, PA; Windber Medical Center, Windber, PA; Concurrent Technologies Corporation, Johnstown, PA; Walter Reed National Military Medical Center, Bethesda, MD

Obesity is a major risk factor for cardiovascular (CV) disease. Behavioral lifestyle change is the cornerstone of therapy for weight management. Currently little is known about molecular responses accompanying weight loss that may be important in weight control and CV risk reduction.

Patients (n=89) participated in a prospective, nonrandomized, lifestyle change program designed to stabilize or reverse progression of CV disease through dietary changes, exercise, and stress reduction. Nonintervention controls (n=63) were matched to patients based on age, gender, and disease status. CV risk factors (BMI, blood pressure, lipids) and peripheral blood gene expression profiles were assessed at three time points over one year.

Most patients were obese (63%; BMI$\geq$30) or overweight (25%; 25$\leq$BMI<30 kg/m^2) at baseline, but showed significant improvement in CV risk factors compared to controls during the program. Following stratification based on weight loss, we observed significant expression changes (FDR P<0.05) for 41 genes in participants who lost the most weight (mean weight loss=11%) from baseline to three months and for 3223 genes in those who lost the most weight (mean weight loss=15%) from baseline to one year. No significant expression changes were observed in patients who lost the least weight (mean weight loss<4%) or in controls. Functional ontologies of genes showing the most significant changes in expression included immune/defense response and symbiosis at three months and metabolism/biosynthesis at one year.

Intensive lifestyle modification can effectively alter CV risk factors, but successful weight loss may accentuate molecular change. Defining the role of weight loss in molecular response to lifestyle modification provides another dimension to understanding complex biological processes involved in CV health.

Presented as poster for Obesity 2012: 30th Annual Scientific Meeting, San Antonio, TX, 20-24 Sep 12.

Novel Stress Reduction Technique Improves Sleep and Fatigue

Mariam Kashani, Arn Eliasson, Karla Bailey, Marina Vernalis

Purpose: A growing body of evidence substantiates the important roles of stress and sleep in cardiovascular disease. We sought to determine the effect of a brief, portable stress reduction technique, the ten-minute Tension Tamer on improvement of stress levels and sleep parameters in a heart health program.
Methods: Adult men and women self-referred or referred to the Integrative Cardiac Health Project were assessed for levels of perceived stress and sleep quality using validated surveys. Subjective stress was measured using the Perceived Stress Scale (PSS14, total possible points 56); sleep quality was evaluated with the Pittsburgh Sleep Quality Index (PSQI, total possible points 21); fatigue was assessed using the 10 point fatigue scale. After a 30-minute introductory workshop, subjects were given instruction and guided opportunities to practice ten-minute Tension Tamers over the course of four 30-minute visits with a stress management specialist. This brief technique, encouraged at bedtime, involves deep breathing and imagery using the subject's personal preference. Upon completion of the four visit practice sequence, validated surveys were reassessed and compared with baseline values using t-tests.
Results: Of 334 subjects (mean age 55.7 years, 135 men, 200 Caucasian, 117 African-American, 14 Latino, 3 other), 218 (65%, mean age 56.6 years, 40% men) improved their perceived stress by 6.6 points ($p<0.001$) using the Tension Tamer technique. Non-improvers, 116 subjects (34%, mean age 59.7, 41% men) showed worsened stress levels by 4.6 points. Comparing Improvers with Non-Improvers showed significant differences in sleep quality (PSQI improved 1.78 vs worsened 0.89 points, $p<0.001$), decreased sleep latency (decreased 4 vs increased 1.9 minutes, $p=0.04$), and decreased fatigue (decreased 0.89 vs increased 0.27 points, $p<0.001$).
Conclusion: A novel stress reduction technique, the ten-minute Tension Tamer, can reduce perceived stress levels in a majority of subjects resulting in improved sleep quality, decreased sleep latency and improved fatigue.
Clinical Implications: Using a portable stress reduction technique in short intervals may be a unique approach to improve cardiovascular risk through sleep improvement.

Accepted for podium presentation at CHEST, Altanta, GA, 22 Oct 12.

CIMT Imaging Knowledge Effect on Lifestyle Program Adherence

Modlin RE, Walizer EM and Vernalis MN

Introduction: The use of carotid intima media thickness (CIMT) ultrasound to identify subclinical atherosclerosis is widespread, but few studies examine its influence on patient behavior. We evaluated the use of CIMT imaging knowledge to motivate adherence to a lifestyle program.

Hypothesis: We hypothesized that participants with cardiovascular disease (CVD) risk factors who have knowledge of their CIMT test results will demonstrate better program adherence than those participants from whom the CIMT test information was withheld.

Methods: Participants, with ≥ 2 CVD risk factors and CIMT measurements ≥ 75th percentile for age, were randomized into either the intervention group [receive results (R-CIMT)] or control group [withhold results (W-CIMT)]. The R-CIMT group received their CIMT image weekly. All participants received the 12-week program (Mediterranean diet, aerobic exercise, group support). We determined the overall change in program adherence from baseline to week 12 or last observation carried forward using an ANCOVA model with CIMT group and gender as factors and age as the covariate. Percent adherence was calculated as a composite measure of diet and exercise adherence at baseline and 12 weeks [Diet adherence = (Mediterranean Diet Score/14) X 100% and Exercise adherence = (weekly exercise time/180) X 100%]. Adherence measures were capped at 100%. R-CIMT group received a CIMT tutorial explaining results and associated CVD risk. Comprehension was assessed by a knowledge test.

Results: 161 participants (mean age=53.6 ± 10.8; 62% women; 48% black) were enrolled over 2 years. No differences were seen between groups in baseline demographics, except W-CIMT group was younger (52 vs. 55 yrs; p=0.049). When comparing R-CIMT vs. W-CIMT groups, no difference was detected in overall % change in adherence (16.4 ± 25.6 vs. 19.8 ± 25.4; p=0.392). The median knowledge test score was 90% (80,100) in the participants responding (66%).

Conclusions: In conclusion, although the presence of subclinical atherosclerosis increased participant knowledge of their increased CVD risk, it did not motivate participants to make more lifestyle changes than those in the control group.

Accepted for Podium Presentation, TriService ACP Meeting, Bethesda, MD, 1-3 Nov 12.

Cardiovascular Disease Risk Factor Modification Decreases HS-CRP and Macrophage Migration Inhibitory Factor (MIF): Influence of Gender

Edward J. Miller, Kimberly A. Mamula, Lin Leng, Marta Piecychna, Marina N. Vernalis, Richard Bucala*, Darrell L. Ellsworth*
*Co-senior authors

Inflammation and gender are key factors in cardiovascular disease (CVD) pathogenesis and outcomes. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that contributes to CVD risk through inflammatory vulnerable plaque formation, while CRP is a systemic marker of inflammation. Lifestyle modification programs focusing on nutrition, exercise, and stress management are effective in mediating CVD risk through traditional measures like weight, blood pressure, and lipids; however, little is known about gender-related differences and response of emerging risk factors such as MIF during lifestyle modification.

In a prospective study of patients with elevated CVD risk matched to controls by age, gender and CVD risk factors (n=85/group), we investigated 1) changes in circulating MIF and HS-CRP, 2) the influence of gender on changes in MIF and HS-CRP, 3) correlation between changes in MIF and HS-CRP during an intensive CVD risk reduction program.

Baseline MIF and HS-CRP were higher in women vs. men (P=0.04) in patients enrolled in the lifestyle modification program (MIF: 3.1 ± 1.9 vs. 2.8 ± 1.9 ng/ml; HS-CRP: 5.9 ± 7.7 vs. 3.5 ± 2.7 ng/ml) and controls (MIF: 3.3 ± 2.0 vs. 2.5 ± 1.7 ng/ml; HS-CRP: 3.7 ± 3.6 vs. 1.6 ± 2.0 ng/ml). After 3 months of lifestyle modification, female gender accounted for the majority of decrease in MIF and HS-CRP. Women showed a 23% decrease in MIF (3.1 ± 1.9 vs. 2.4 ± 1.2 ng/ml, P=0.05) and a 40% decrease in HS-CRP (5.9 ± 7.7 vs. 3.5 ± 4.5 ng/ml, P=0.06), but neither MIF nor HS-CRP changed significantly in controls or men in the lifestyle modification program. Pair wise correlation did not show a relationship between changes in MIF and HS-CRP.

In summary, pro-inflammatory MIF and HS-CRP decreased in response to intensive diet/lifestyle intervention, with improvement being more evident in women than men. While changes in weight and blood pressure were similar in both genders during the lifestyle intervention, changes in inflammatory markers were dependent on gender. This suggests intensive lifestyle modification may lessen CVD risk in women through different mechanisms than in men.

Accepted for poster presentation at AHA Scientific Session 2012, Los Angeles, CA, 3-7 Nov 12.

SNPs associated with plasma triglyceride levels in the general population influence response during intensive cardiovascular risk reduction

Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL

Integrative Cardiac Health Program, Windber Research Institute, Windber, PA; Windber Medical Center, Windber, PA, USA; Integrative Cardiac Health Program, Walter Reed National Military Medical Center, Bethesda, MD

Background: Triglycerides are lipid fractions that represent an important risk factor for cardiovascular disease (CVD) because they play a fundamental role in development and progression of atherosclerosis. Although current guidelines advocate lifestyle change involving diet, physical activity, and weight control for management of hypertriglyceridemic patients, plasma triglyceride levels may be influenced by genetic composition in addition to lifestyle behaviors. Recent genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) associated with plasma triglyceride levels in the general population.

Methods: We examined the influence of genetic variation on variability in triglyceride response in 178 participants who completed a prospective, non-randomized intervention designed to stabilize or reverse progression of CVD through dietary changes, exercise, and stress reduction. Cardiovascular risk factors were assessed at baseline, 12 weeks, and 52 weeks by standard methods. SNPs (n=19) associated with plasma triglycerides were genotyped by TaqMan® allelic discrimination assays.

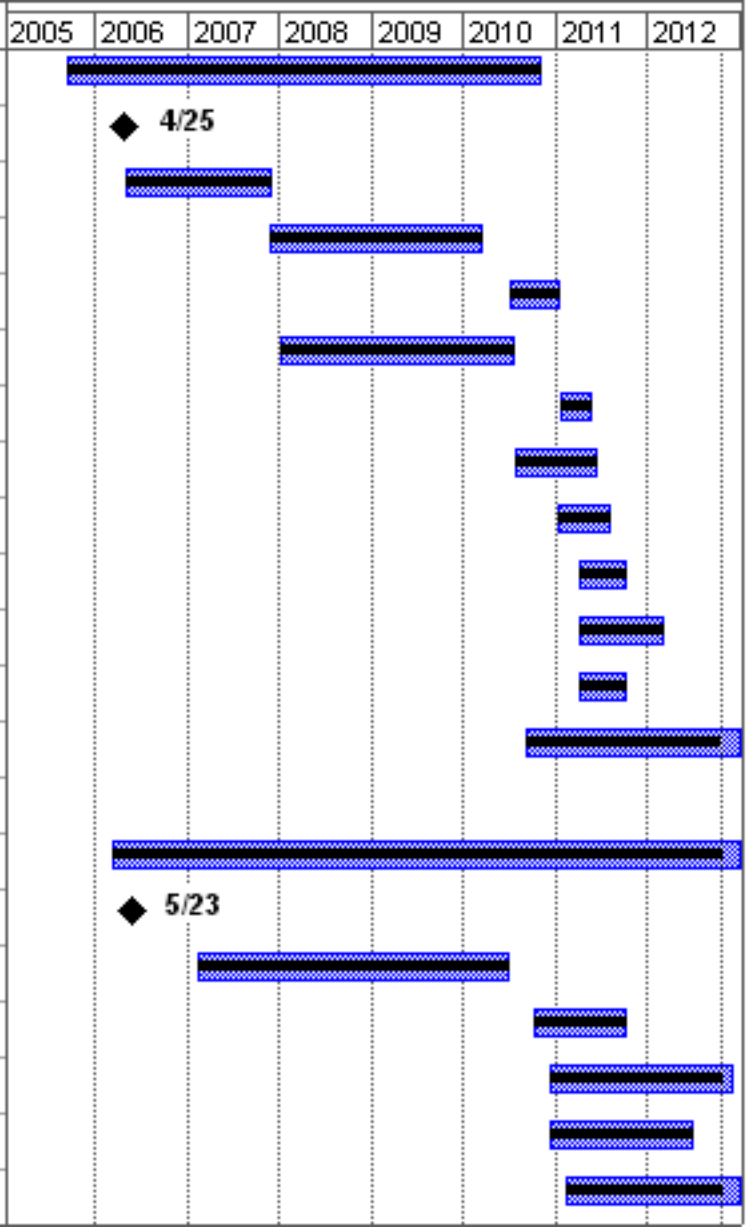
Results: Patients experienced significant improvement ($P < 0.05$) in most risk factors, including weight (-9%), blood pressure (-6%), total cholesterol (-7%), and triglycerides (-9%). Triglyceride response during the program differed significantly ($P < 0.05$) between genotypes for three SNPs (rs442177, rs3846662, and rs17145738) located close to the following genes: transcriptional activator AF4/FMR2 family member 1 (AFF1), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), which catalyzes the rate-limiting step in cholesterol synthesis, and MLX interacting protein-like (MLXIPL), which controls transcription of genes involved in glycolysis.

Discussion: Lifestyle modification for cardiovascular risk reduction may be more beneficial to certain individuals based on genetic composition. Genetic variation associated with CVD risk may provide a basis for personalized treatments to optimize cardiovascular health.

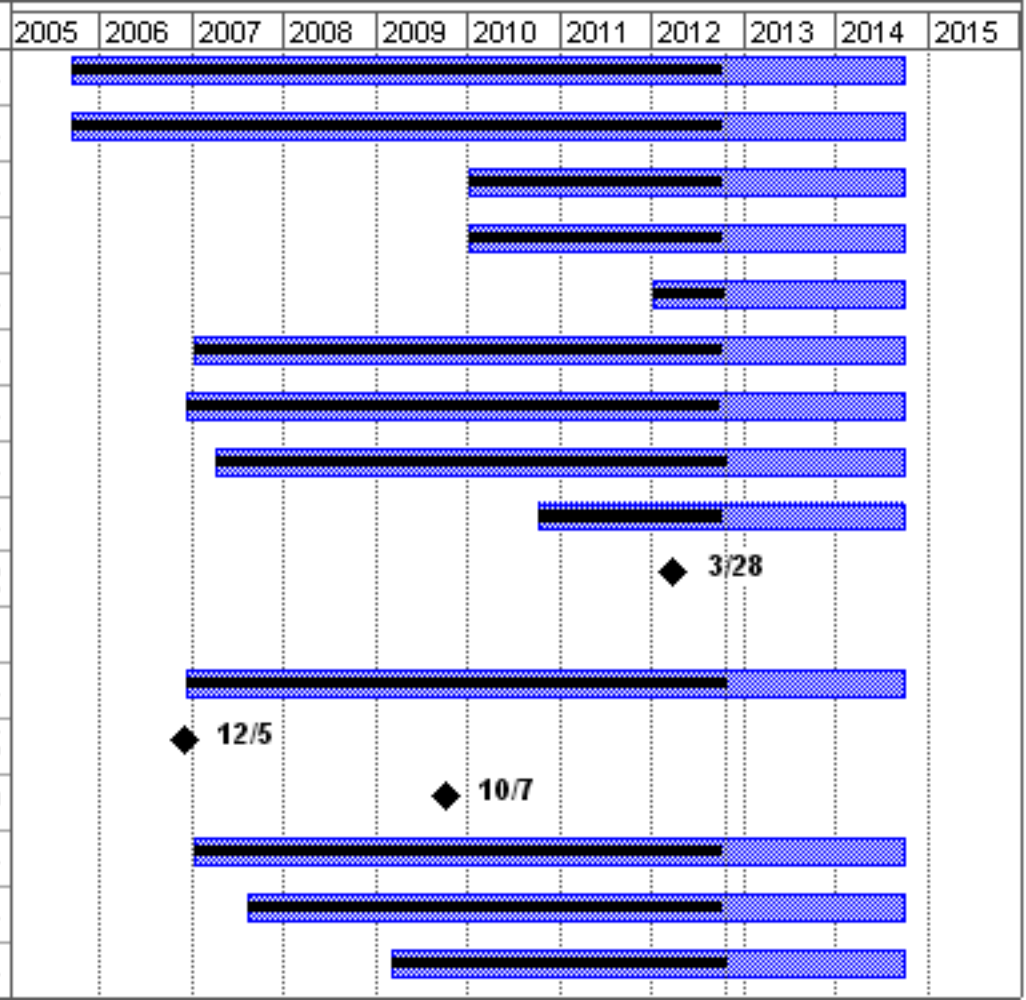
Accepted for poster presentation at the ASHG Meeting, San Francisco, CA, 6-10 Nov 12.

Appendix B
Gantt Charts

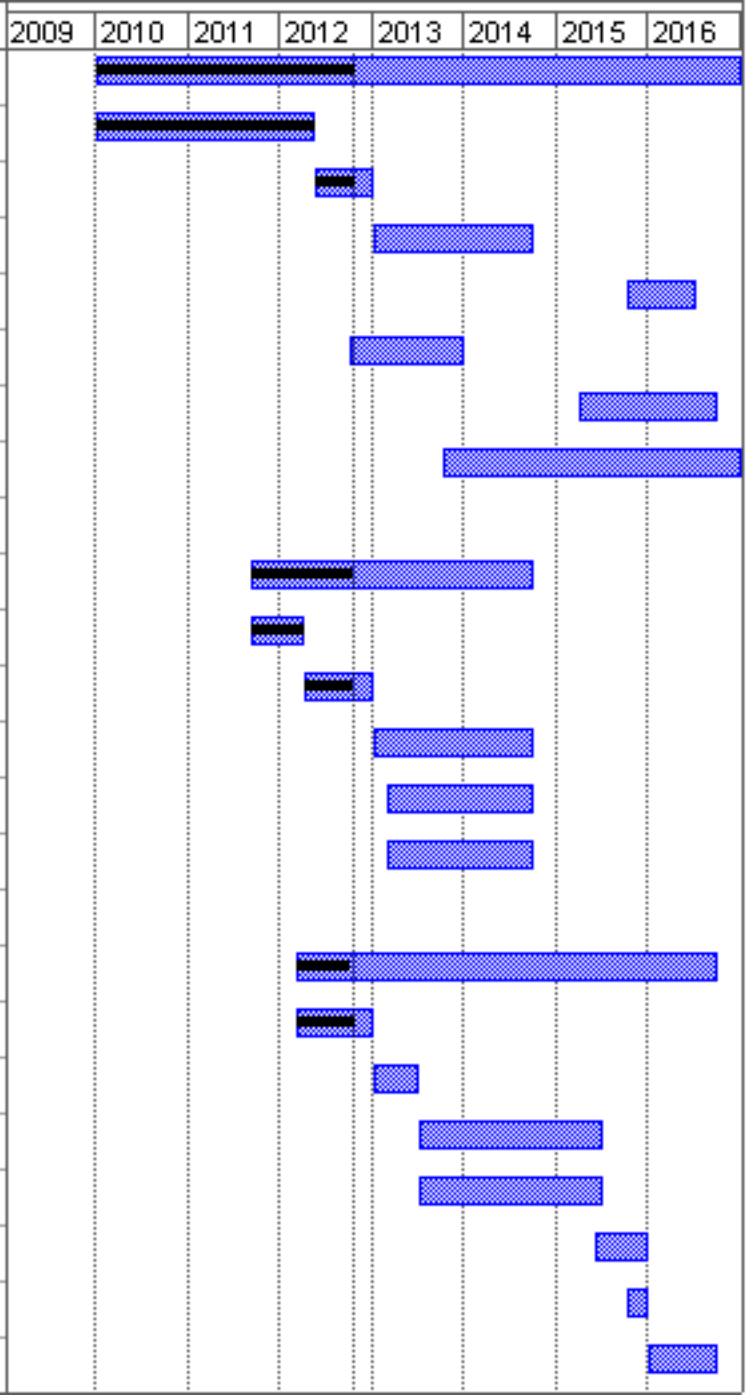
ID	Task Name	Start	Finish
1	Task #1: BATTLE trial	Thu 9/1/05	Fri 10/29/10
2	IRB protocol approval	Tue 4/25/06	Tue 4/25/06
3	Intervention preparation	Tue 4/25/06	Fri 11/30/07
4	Recruitment/Enrollment/Data	Thu 11/15/07	Wed 3/10/10
5	Addendum submission/approval	Thu 7/1/10	Fri 1/14/11
6	Data collection (Main study)	Tue 1/1/08	Thu 7/15/10
7	Data collection (Addendum)	Tue 1/18/11	Wed 5/18/11
8	Database reconciliation (Main study)	Thu 7/15/10	Wed 6/15/11
9	Data analysis (Main study)	Mon 1/3/11	Fri 7/29/11
10	Quantitative analysis (Addendum)	Fri 4/1/11	Fri 9/30/11
11	Qualitative analysis (Addendum)	Fri 4/1/11	Tue 2/28/12
12	Publication plan	Fri 4/1/11	Fri 9/30/11
13	Presentations and manuscripts	Wed 9/1/10	Mon 12/31/12
14			
15	Task #2: CADRe Five-Year Follow-up	Wed 3/1/06	Mon 12/31/12
16	IRB protocol approval	Tue 5/23/06	Tue 5/23/06
17	Participant enrollment/Data collection	Fri 2/2/07	Wed 6/30/10
18	Data reconciliation	Fri 10/1/10	Fri 9/30/11
19	Conduct analysis	Wed 12/1/10	Fri 11/30/12
20	Publication plan	Wed 12/1/10	Fri 6/29/12
21	Presentations and manuscripts	Tue 2/1/11	Thu 1/31/13



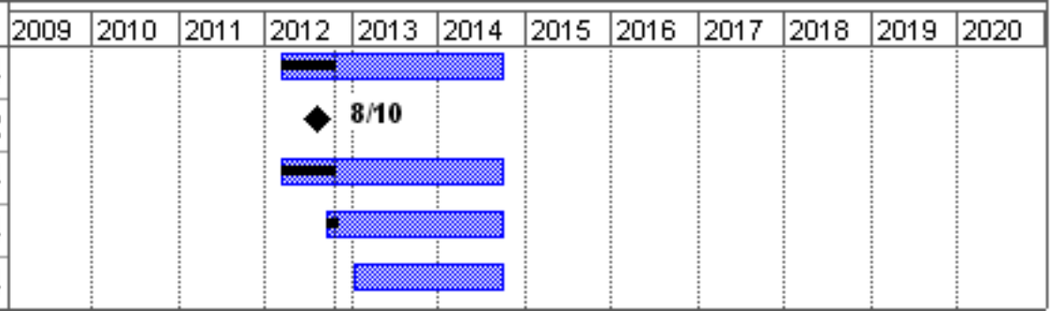
ID	Task Name	Start	Finish
1	Task #3: Continue CPP	Thu 9/1/05	Tue 9/30/14
2	Enrollment/Data collection	Thu 9/1/05	Tue 9/30/14
3	Advance data modeling	Fri 1/1/10	Tue 9/30/14
4	Outreach	Fri 1/1/10	Tue 9/30/14
5	Ultra personal empowering	Mon 1/2/12	Tue 9/30/14
6	Outcomes analysis	Mon 1/1/07	Tue 9/30/14
7	Target subgroup popns	Fri 12/1/06	Tue 9/30/14
8	Presentations/manuscripts	Mon 4/2/07	Tue 9/30/14
9	Upgrade database	Fri 10/1/10	Tue 9/30/14
10	Retro CPP Outcomes CR	Wed 3/28/12	Wed 3/28/12
11			
12	#3.1: Validate CV risk	Tue 12/5/06	Tue 9/30/14
13	IRB protocol approval	Tue 12/5/06	Tue 12/5/06
14	Continuing review approved	Wed 10/7/09	Wed 10/7/09
15	Data collection	Mon 1/1/07	Tue 9/30/14
16	Conduct analysis	Wed 8/1/07	Tue 9/30/14
17	Presentations/manuscripts	Mon 3/2/09	Tue 9/30/14



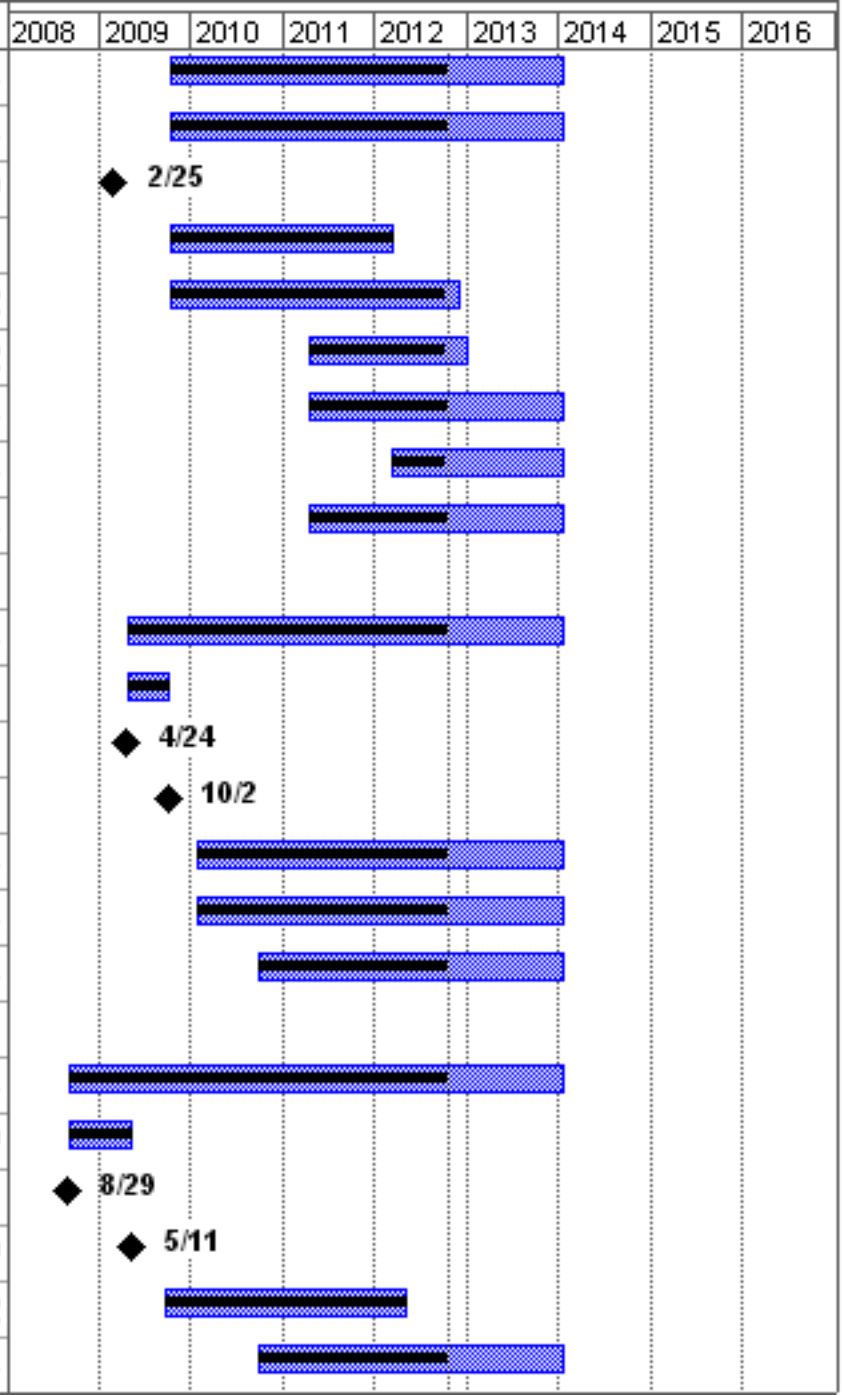
ID	Task Name	Start	Finish
1	Subtask #3.2: Initiate ZENITH trial	Fri 1/1/10	Fri 12/30/16
2	Protocol development/submission	Fri 1/1/10	Wed 5/9/12
3	Protocol approval	Thu 5/10/12	Mon 12/31/12
4	Recruitment/enrollment/data collection	Tue 1/1/13	Tue 9/30/14
5	Conduct analysis	Thu 10/1/15	Thu 6/30/16
6	Conduct molecular analysis	Mon 10/1/12	Tue 12/31/13
7	Publication plan	Wed 4/1/15	Fri 9/30/16
8	Presentations and manuscripts	Tue 10/1/13	Fri 12/30/16
9			
10	Subtask #3.3: CPP Prospective Registry	Thu 9/1/11	Tue 9/30/14
11	Protocol development/submission	Thu 9/1/11	Fri 3/30/12
12	Protocol approvals	Mon 4/2/12	Mon 12/31/12
13	Data collection	Tue 1/1/13	Tue 9/30/14
14	Data reconciliation/analysis	Fri 3/1/13	Tue 9/30/14
15	Manuscript preparation	Fri 3/1/13	Tue 9/30/14
16			
17	Task #4: LEASE Trial	Thu 3/1/12	Fri 9/30/16
18	Protocol development/submission	Thu 3/1/12	Mon 12/31/12
19	Protocol approvals	Tue 1/1/13	Fri 6/28/13
20	Recruitment/enrollment/data collection	Mon 7/1/13	Wed 7/1/15
21	Molecular studies	Mon 7/1/13	Wed 7/1/15
22	Data reconciliation/analysis	Mon 6/1/15	Thu 12/31/15
23	Publication plan	Thu 10/1/15	Thu 12/31/15
24	Manuscript preparation	Fri 1/1/16	Fri 9/30/16



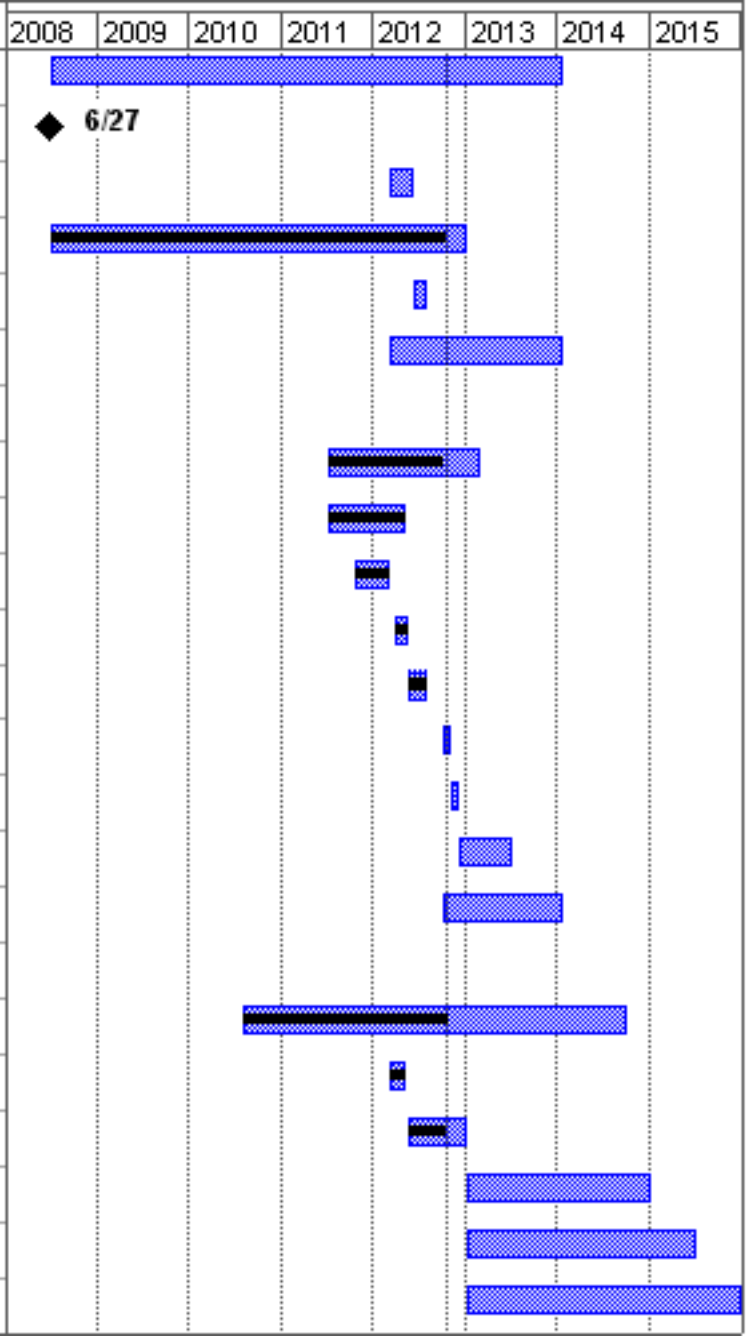
ID	Task Name	Start	Finish
1	Subtask #3.4: CV Risk in Tramatic Amputations	Thu 3/1/12	Tue 9/30/14
2	Protocol approval	Fri 8/10/12	Fri 8/10/12
3	Recruitment/enrollment/data collection	Thu 3/1/12	Tue 9/30/14
4	Interim data analysis	Mon 9/3/12	Tue 9/30/14
5	Presentations and manuscripts	Tue 1/1/13	Tue 9/30/14



ID	Task Name	Start	Finish
1	Task #1: Global Profiling	Thu 10/1/09	Tue 1/21/14
2	Followup data analysis/publication	Thu 10/1/09	Tue 1/21/14
3	Enroll program participants	Wed 2/25/09	Wed 2/25/09
4	Manuscript on insulin and leptin	Thu 10/1/09	Fri 3/16/12
5	Manuscript on gene expression	Thu 10/1/09	Fri 11/30/12
6	TaqMan SNP analysis	Thu 4/14/11	Mon 12/31/12
7	Metabolite profiling analysis	Thu 4/14/11	Tue 1/21/14
8	Assimilation of PET/CT data	Thu 3/1/12	Tue 1/21/14
9	Presentations & publications	Thu 4/14/11	Tue 1/21/14
10			
11	Task #2: Cardiovascular Risk Clinic	Fri 4/24/09	Tue 1/21/14
12	IRB protocol development	Fri 4/24/09	Fri 10/2/09
13	Protocol approved - WMC	Fri 4/24/09	Fri 4/24/09
14	Protocol approved - TATRC	Fri 10/2/09	Fri 10/2/09
15	Enroll program participants	Tue 1/19/10	Tue 1/21/14
16	Enroll control subjects	Tue 1/19/10	Tue 1/21/14
17	Conduct molecular analysis	Wed 9/15/10	Tue 1/21/14
18			
19	Subtask #2.1: STEP	Fri 8/29/08	Tue 1/21/14
20	IRB protocol development	Fri 8/29/08	Mon 5/11/09
21	Protocol approved at WMC	Fri 8/29/08	Fri 8/29/08
22	Protocol approved at TATRC	Mon 5/11/09	Mon 5/11/09
23	Enroll program participants	Tue 9/15/09	Mon 4/30/12
24	Conduct molecular analysis	Wed 9/15/10	Tue 1/21/14



ID	Task Name	Start	Finish
1	Task #3: MI in Young Military Personnel	Fri 6/27/08	Tue 1/21/14
2	Protocol approved at WMC	Fri 6/27/08	Fri 6/27/08
3	Protocol approved at TATRC	Thu 3/1/12	Thu 5/31/12
4	Develop molecular methods	Mon 6/30/08	Mon 12/31/12
5	Query MHS EHR to identify samples	Fri 6/1/12	Tue 7/31/12
6	Conduct molecular analysis	Thu 3/1/12	Tue 1/21/14
7			
8	Task #4: DODSR Proof of Principle	Fri 7/1/11	Thu 2/28/13
9	Protocol development/submission	Fri 7/1/11	Tue 5/1/12
10	Protocol approved at WRNMMC	Fri 10/14/11	Mon 3/5/12
11	Protocol approved at WMC/Vanderbilt	Mon 3/26/12	Sun 5/13/12
12	Protocol approved at TATRC	Mon 5/14/12	Tue 7/31/12
13	Retrieve appropriate samples	Mon 10/1/12	Tue 10/30/12
14	Ship de-identified samples to WRI	Thu 11/1/12	Fri 11/30/12
15	Conduct molecular analysis	Mon 12/3/12	Fri 6/28/13
16	Presentations and manuscripts	Mon 10/1/12	Tue 1/21/14
17			
18	Task #5: Natural History of Pre-Diabetes	Mon 8/2/10	Tue 9/30/14
19	Protocol development/submission	Thu 3/1/12	Mon 5/7/12
20	Protocol approvals	Thu 5/10/12	Mon 12/31/12
21	Recruitment/enrollment/data collection	Wed 1/2/13	Wed 12/31/14
22	Conduct analyses	Tue 1/1/13	Tue 6/30/15
23	Presentations and manuscripts	Tue 1/1/13	Thu 12/31/15



ID	Task Name	Start	Finish
1	Task #6: Molecular Biology Morbid Obesity	Fri 1/13/06	Tue 1/21/14
2	Protocol approved at WMC	Fri 1/13/06	Fri 1/13/06
3	Protocol approved at TATRC	Fri 6/15/12	Fri 6/15/12
4	Enroll patients	Mon 7/24/06	Thu 2/28/13
5	Obtain blood and tissue samples	Mon 7/24/06	Thu 2/28/13
6	Conduct molecular analysis	Mon 10/1/12	Fri 5/31/13
7	Presentations and manuscripts	Tue 1/1/13	Tue 1/21/14